ETIOPATHOLOGICAL STUDY OF PANCYTOPENIA – AN INSTITUTIONAL STUDY

By

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PATHOLOGY

Under the Guidance of

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ABBREVATIONS

AIDS - Acquired immunodeficiency syndrome

ALL - Acute lymphoid leukemia

AML Acute myeloid leukemia

BM - Bone marrow

BMA/ BMB - Bone marrow aspiration/ Bone marrow biopsy

BFU-E Burst Forming Unit - Erythroid

CFU – GM - Colony forming unit – Granulocyte - Macrophage

CFU-E - Colony forming unit-Erythroid

CFU-S - Colony forming unit-Spleen

CLL - Chronic lymphocytic leukemia

DNA - Deoxyribonucleic acid

FAB - French American British

GPI - Glycosyl Phosphatidyl Inositol

HB - Hemoglobin

HE - Hereditary Elliptocytosis

HLA - Human leucocyte antigen

MCV - Mean Cell Volume

MDS – Myelodysplastic syndrome

M: E - Myeloid Erythroid Ratio

NHL - Non Hodgkin lymphoma

PCR - Polymerase chain reaction

PBS – Peripheral blood smear

PNH Paroxysmal nocturnal hematuria

PRCA – Pure red cell aplasia

RAEB-t Refractory anemia with excess blasts in transformation

RARS Refractory anemia with ringed sideroblasts

SLE Systemic lupus erythematosus

TLC – Total leucocyte count

WBC - White Blood Count

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ABSTRACT

ETIOPATHOLOGICAL STUDY OF PANCYTOPENIA: AN INSTITUTIONAL STUDY

Background: Pancytopenia is a common hematological problem characterized by anemia, leucopenia and thrombocytopenia,. It is a striking feature of many serious and life threatening illnesses. The disease pattern varies in different population groups, in age pattern, nutritional status and prevalence of infective disorder. Present study was conducted to assess the etiology, clinical profile and bone marrow morphology of pancytopenia.

Objectives:

1) To study the clinical presentations and hematological parameters in patients with pancytopenia.

2) To study the morphological pattern in bone marrow in patients with pancytopenia.

Methods:

A two year study was conducted in Department of Pathology, Sri Devaraj Urs medical college, Kolar, Karnataka. Total 70 pancytopenia patients were studied to determine their presenting symptoms and signs, peripheral blood smear study and bone marrow morphology.

Results:

Bone marrow study showed 48.5% hypercellular marrow, 32.8% normocellular and 18.7 % hypocellular marrow. Females(55.7%) were affected more than males(44.2%) and the commonest cause for pancytopenia was megaloblastic anemia (51.4%), followed by iron deficiency anemia (37.1%),viral infections(4.2%),leukemia (1.4%), MDS (2.8%), pure red cell aplasia (1.4%), hereditary elliptocytosis (1.4%).

Conclusion: Large number of patients with pancytopenia had reversible etiology. Hence complete work up including clinical details with hematological examination along with bone marrow study will lead to early and proper diagnosis of case followed by proper treatment.

Key words: Pancytopenia; Megaloblastic anemia.

INTRODUCTION

Bone marrow is the largest and most widely distributed organ in the body. It is the principle site for blood cell formation. Sir William Harvey described blood as "the fountain of life and the primary seat of the soul. The marrow of our bones is the seedbed of our blood".[1] In the normal adult, its daily production and export of blood cells amounts to about 2.5 million red cells, 2.5 billion platelets and 1.0 billion granulocytes per kilogram of body weight.

Cytopenia is a disorder in which production of one or more blood cell types ceases or is greatly reduced.

The spectrum of disorders primarily or secondarily affecting the bone marrow may manifest with peripheral pancytopenia. Pancytopenia is a disorder in which all three major formed elements of blood (red blood cells, white blood cells and platelets) are decreased than normal.

It is not a disease entity, but a triad of findings that may result from a number of disease processes–primarily or secondarily involving the bone marrow.[2] The presenting symptoms are often attributable either to the anemia or thrombocytopenia. Anemia leads to fatigue, dyspnea and cardiac symptoms. Thrombocytopenia leads to bruising and mucosal bleeding. Neutropenia leads to leucopenia seen in the subsequent course of the disorder.

Many hematopoietic and non-hematopoietic conditions manifest with features of pancytopenia. Pancytopenia is a striking feature of many serious and life threatening illnesses ranging from simple drug induced bone marrow hypoplasia, megaloblastic marrow to fatal bone marrow aplasias and leukemias.

The pattern of diseases leading to pancytopenia is expected to vary in different population groups with their difference in age pattern, nutritional status and prevalence of the infective disorder.

The mechanisms contributing to pancytopenia include, decrease in hematopoietic cell production, marrow replacement by abnormal cells, suppression of marrow growth and differentiation, ineffective hematopoiesis with cell death, defective cell formation, antibody mediated sequestration or destruction of cells in a hypertrophied and overactive reticuloendothelial system.[3]

Careful assessment of the blood elements is often the first step in assessment of hematologic function and diagnosis of disease. Physical findings and peripheral blood picture provide valuable information in the work up of pancytopenia patients and help in planning investigations on bone marrow samples.

Bone marrow evaluation is an invaluable diagnostic procedure in practice of medicine which may confirm the diagnosis of suspected cytopenia, from the clinical features and peripheral blood examination or occasionally give a previously unsuspected diagnosis.[4]

Bone marrow aspiration establishes the diagnosis, however if the tap is dry, then bone marrow biopsy becomes mandatory for the diagnosis. Aspiration smears are superior for morphological details, while biopsy provides a more reliable index of cellularity and often reveals bone marrow infiltration, fibrosis and granulomas.

The severity of Pancytopenia and the underlying pathology determine the management and prognosis of these patients.

Previous studies done in India, stress the important of megaloblastic anemia as being major cause of pancytopenia.[4,5] Present study has been undertaken to evaluate the etiology, clinical profile and bone marrow morphology of pancytopenia. Thereby, this data would help in planning the diagnostic and therapeutic approach in patients with pancytopenia.

OBJECTIVES

1) To study the clinical presentations and hematological parameters in patients with pancytopenia.

2) To study the morphological pattern in bone marrow in patients with pancytopenia.

REVIEW OF LITERATURE

Until the 19th century, blood cell formation was thought to be prerogative of the lymph nodes or the liver and spleen. In 1868, Neuman and Bizzozero independently observed nRBCs in material squeezed from the rib of human cadaver and proposed that the marrow is major source of blood cells.[6]

Metcalf & Moore in 1971 observed that red cells, leucocytes and platelets constitute the essential cellular components of the blood. Formation of blood cells occurs at different anatomical sites during the course of the development from embryonic to adult life.[7]

Production of blood cells commences in the yolk sac of the embryo, then shifts to liver and spleen in utero life and then superseded by the bone marrow which serves as the only important site of blood cell production after birth.[7]

In the normal adult, daily marrow production amounts to be approximately 2.5 billion RBC, 2.5 million platelets and 1.1 billion granulocytes per kg of body weight. The rate of production adjusts to actual needs and can vary from zero to many times normal.

The hemopoeitic system is a hierarchy of cells in which multipotent hemopoeitic stem cells give rise to lineage-committed progenitor cells, which divide to generate maturing and mature blood cells. Haematopoiesis begins early during embryogenesis and undergoes many changes during fetal and neonatal development. Unlike some organ systems that form in early life and are not continually replaced, turnover and replenishment of the hematopoietic system continue throughout life.

Peripheral cytopenia is defined as reduction in one of the cellular elements of blood i.e. red cells, white cells and platelets. Bicytopenia is reduction in any of the two cell lines and pancytopenia is the reduction in all three elements.

Pancytopenia is not an uncommon haematological finding encountered in our clinical practice and should be suspected on clinical grounds when a patient presents with unexplained anemia, prolonged fever and tendency to bleed.

Anemia is present when the hemoglobin level in the blood is below the lower extreme of the normal range for the age and sex of the individual.

Thrombocytopenia is defined as a reduction in the peripheral platelet count below the lower limit of $100X10^9/L$.

Leucopenia is defined as a reduction in the Total leucocyte count below the lower limit of $4X10^9/L$.

ETIOLOGY OF PANCYTOPENIA

1. PANCYTOPENIA WITH HYPOCELLULAR MARROW

- ACQUIRED APLASTIC ANEMIA

A. IDIOPATHIC

B. SECONDARY

a) Irradiation

b) Drugs and Chemicals - Chloramphenicol, NSAIDs

c) Viruses - EBV, Parvovirus B 19, Hepatitis, HIV

d) Immune disorders

i. Eosinophilic fasciitis

ii. Hypoimmunoglobulinemia

iii. Thymoma / Thymic carcinoma

iv. Graft vs Host disease in immunodeficiency

v. Paroxysmal Nocturnal Hemoglobinuria

vi. Pregnancy

- INHERITED

a) Fanconi‟s anemia

b) Dyskeratosis Congenita

c) Schwachmann-Diamond syndrome

d) Reticular Dysgenesis

e) Familial aplastic anemia

- SOME MYELODYSPLASIA SYNDROMES
- RARE ALEUKEMIC LEUKEMIA
- SOME ACUTE LYMPHOBLASTIC LYMPHOMAS

2. PANCYTOPENIA WITH CELLULAR BONE MARROW

A. PRIMARY BONE MARROW DISEASES

1) Myelodysplasia syndromes

2) Paroxysmal Nocturnal Hemoglobinuria

3) Myelofibrosis

4) Myelophthisis

5) Bone marrow lymphoma

6) Hairy cell leukemia

B. SECONDARY TO SYSTEMIC DISEASES

1) Systemic lupus erythematosus

2) Sjogren‟s syndrome

3) Hypersplenism

4) B12 and folate deficiency

5) Overwhelming infection

6) Alcohol

7) Sarcoidosis

8) Tuberculosis and Atypical Mycobacteria

3. HYPOCELLULAR BONE MARROW +/- CYTOPENIA

1) Q fever

2) Tuberculosis

3) Mycobacteria

4) Legionnaire's disease

5) Hypothyroidism

ANATOMY OF THE BONE MARROW

The bone marrow is a unique microenvironment that supports the orderly proliferation, differentiation and release of blood cells. It is filled with a network of thin walled sinusoids lined by a single layer of endothelial cells, which are underlaid by a discontinuous basement membrane and adventitial cells. Within the interstitium lie clusters of hematopoietic cells and fat cells. Differentiated blood cells enter the circulation by transcellular migration through the endothelial cells. The arteriole provides blood supply to the marrow whereas the venous sinus, is a collection system within the marrow where mature cells enter the circulation for the very first time including segmented neutrophils, basophils, eosinophils, red blood cells, monocytes, and platelets. This is how cells enter the general circulation from areas of hematopoiesis in the marrow.

The normal marrow is organized in subtle, but important, ways. For example, normal megakaryocytes lie next to sinusoids and extend cytoplasmic processes that bud off into the blood stream to produce platelets, while red cell precursors often surround macrophages (so called nurse cells) that provide some of the iron needed for the synthesis of hemoglobin. The bone trabeculae provide a network of supportive framework for hematopoiesis to occur. Diseases that distort the marrow architecture, such as deposits of metastatic cancer or granulomatous disease, can cause the abnormal release of immature precursors into the peripheral blood, a finding that is referred to as leukoerythroblastosis.

Bone marrow is semifluid and easily aspirated through a needle. Marrow aspirate smears provide the best assessment of the morphology of hematopoietic cells. The most mature marrow precursors can be identified based on their morphology alone. Immature precursors ('blast' forms) of different types are morphologically similar and must be identified definitively using lineage specific antibodies and histochemical markers. Trephine biopsy is a good means for estimating marrow activity. In normal adults, the ratio of fat cells to hematopoietic elements is about 1:3. The ratio of hematopoietic elements versus fat cells depends on the age and activity of bone marrow in response to various physiologic stimuli. Marrow cellularity is roughly inversely proportion to the age. Thus, newborns have almost 100% cellular bone marrow whereas older individuals in their eighth or ninth decades have 20-30% cellularity. In hypoplastic states (e.g., aplastic anemia) the proportion of fat cells is greatly increased; conversely, fat cells often disappear when the marrow is involved by hematopoietic tumors and in diseases characterized by compensatory hyperplasia (e.g., hemolytic anemias), and neoplastic proliferations such as leukemias. Other disorders (such as metastatic cancers and granulomatous diseases) induce local marrow fibrosis. Such lesions are usually inspirable and best seen in biopsies.

Table 1: Cell composition of aspirated material from a normal adult bone marrow

Erythroid series	Percentage (%)
Pronormoblast	0.1-1.1
Basophilic normoblast	0.4-2.4
Polychromatophilic normoblast	2-30
Orthochromatophilic normoblast	2-10
Granulocytic series	
Myeloblast	0.1-3.5
Promyelocyte	0.5-5
Myelocyte	5-23
Metamyelocyte	7-27
Band form	9-18
Mature Neutrophils	4-28
Lymphocyte	5-24
Plasma cells	0-3.5
Monocytes	0-0.6
Macrophages	0-2
Megakaryocyte	0-0.5

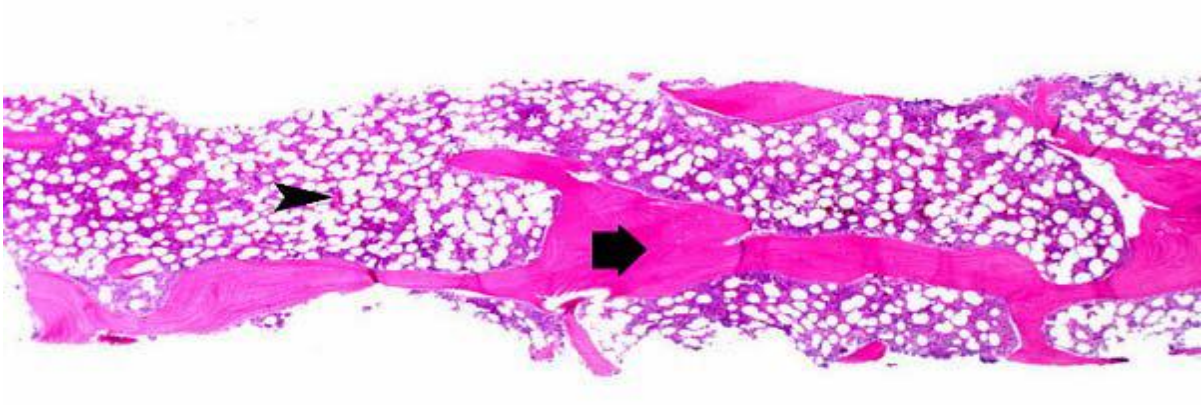


Fig 1: **BMB showing bony spicules, fat spaces and hematopoietic cells. Big arrow – bony trabeculae, small arrow – hematopoietic cells. H & E stain (10x) (Courtesy: curesearch.org)**

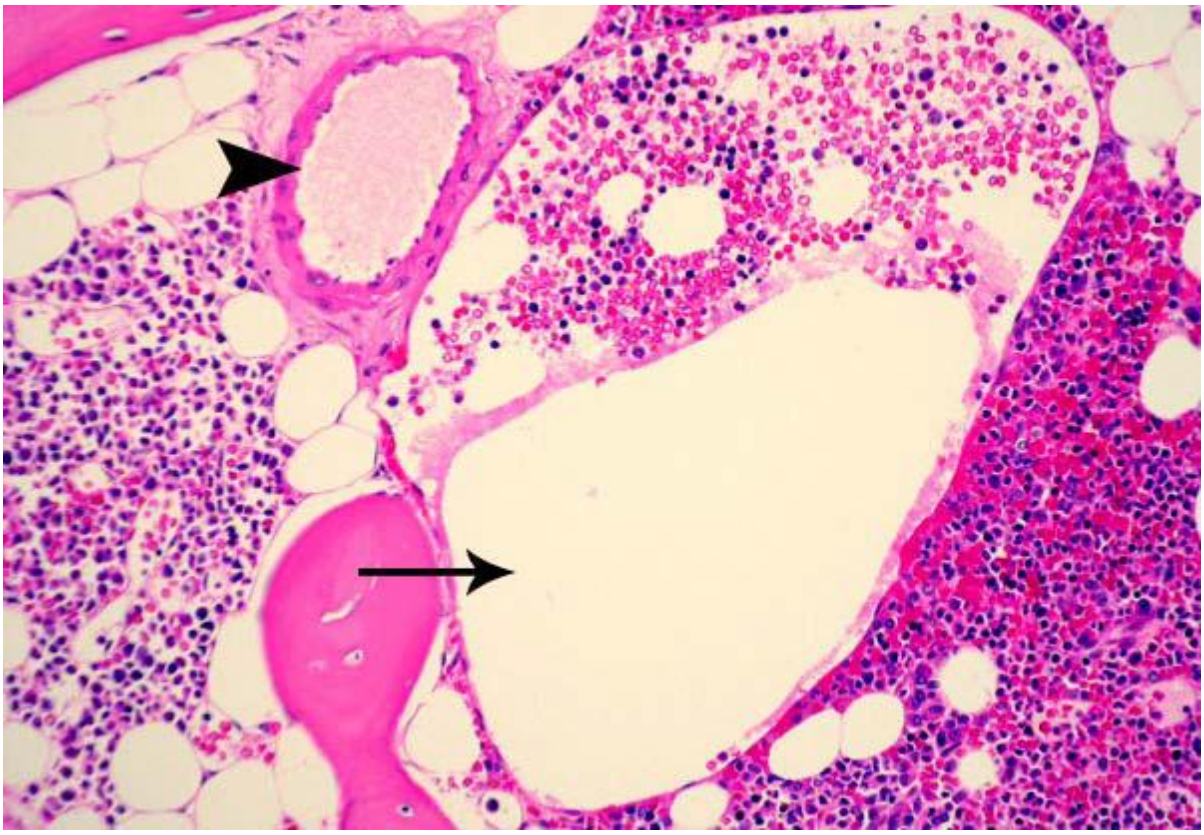


Fig 2: BMB showing blood supply of bone marrow. Small arrow – arteriole, long arrow – venule. H &E stain (10x) (Courtesy: curesearch.org)

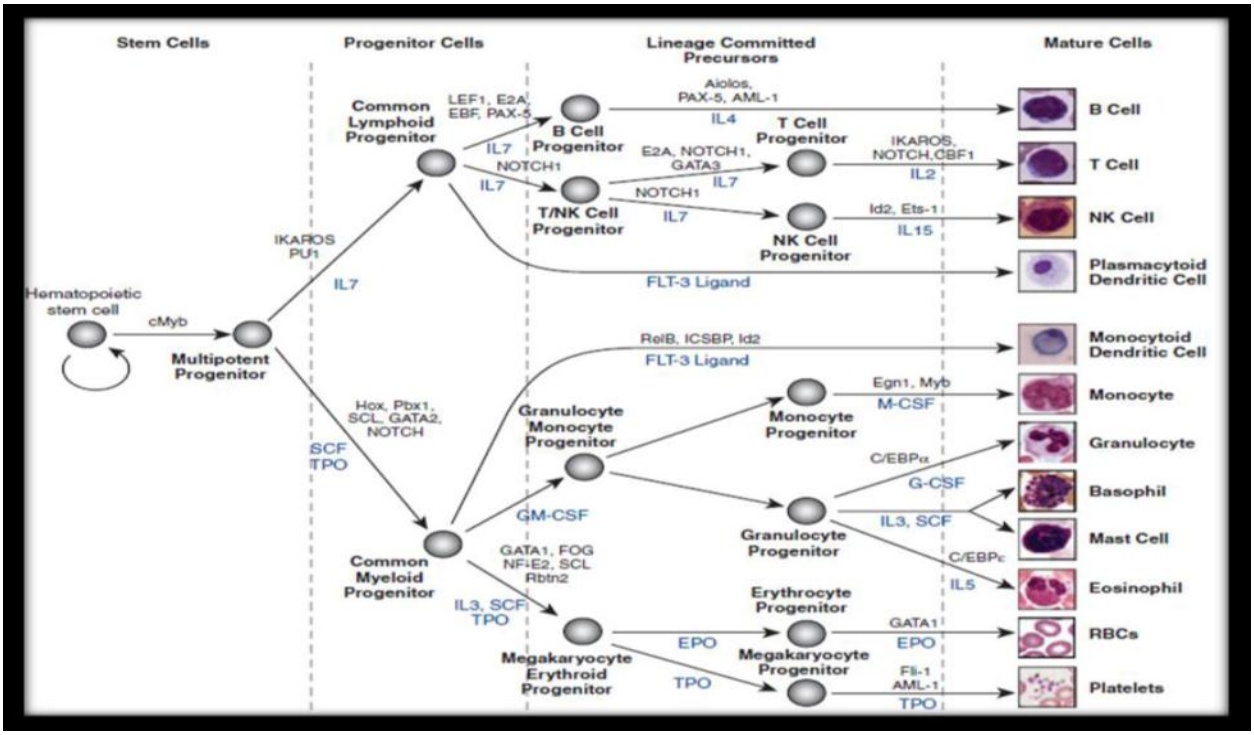


Fig 3: Origin and differentiation of hematopoietic cell (Courtesy: Shirlyn. Mckenzie)

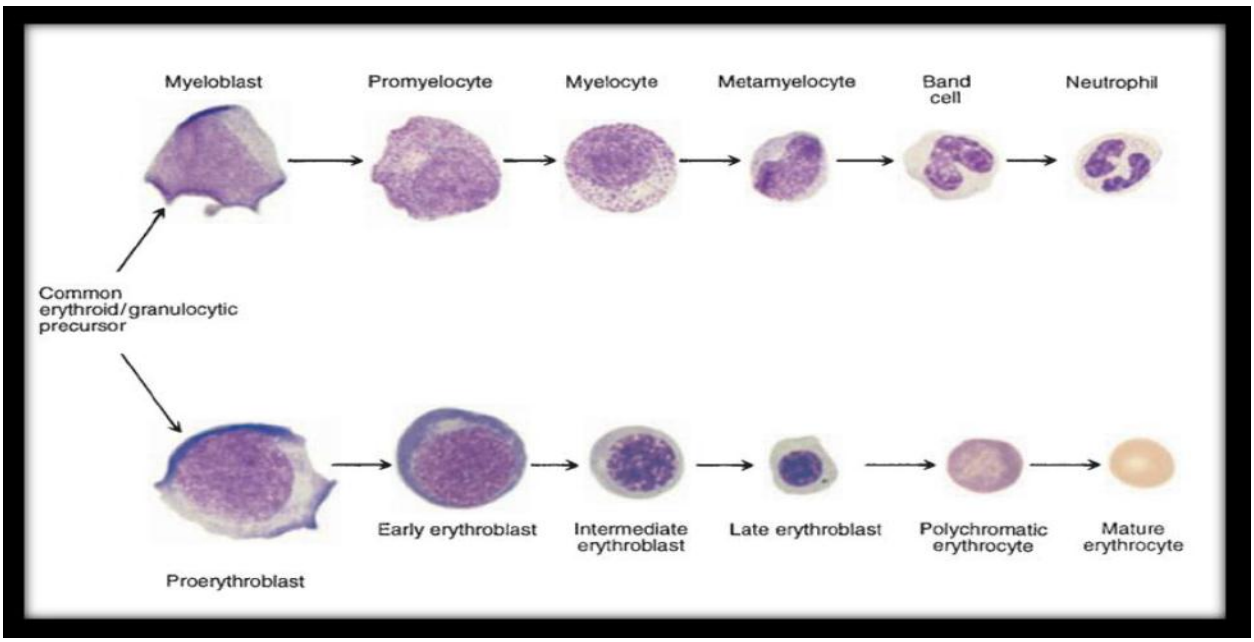


Fig 4: Differentiation of myeloid and erythroid series (Courtesy: Shirlyn. Mckenzie)

HEMOPOIETIC CELLS: ORIGIN AND DIFFERENTIATION

The formed elements of blood - red cells, granulocytes, monocytes, platelets and lymphocytes - have a common origin from pluripotent hemopoetic stem cells. The pluripotent stem cell gives rise to two types of multipotent progenitors, the common lymphoid and common myeloid stem cells. The common lymphoid stem cell in turn gives rise to precursors of T-cells (pro-T cells), B cells (pro B cells) and Natural Killer cells.

From the common myeloid stem cell arise atleast three types of committed stem cells capable of differentiating along the erythroid /megakaryocytic, eosinophilic and granulocyte-macrophage pathways. From the various committed stem cells are derived intermediate stages and ultimately the morphologically recognisable precursors of the differentiated cells, such as proerythroblasts, myeloblasts, megakaryoblasts and monoblasts, which in turn give rise to mature progeny.

1. Regulation of Erythropoiesis

In postnatal life, erythropoiesis takes place within the environment of bone marrow mainly concentrated in axial skeleton, ribs and pelvis. In the steady state, the hematopoietic microenvironment is probably the most important controlling aspect of erythropoiesis, with local cytokine release from the bone marrow and the binding of cytokines to the stromal matrix which determines the rate of proliferation and differentiation. Erythropoietin, produced predominantly by the kidneys in adults, stimulates the conversion of Erythropoietin Responsive Cells to pronormoblasts.

The erythroid progenitor cells can be identified by the characteristics of the erythroid colonies. The earliest recognisable committed progenitor for erythroid cells is the CFU-GEMM (Colony Forming Unit - Granulocytes Erythroid, Megakaryocyte and Macrophage). The next identifiable

progenitor cells are the Burst Forming Units- Erythroid (BFU-E). The final progenitor cell is identified as CFU-E. The Epo-R (Erythropoietin receptor) is expressed on the surface of all the committed erythroid cells in the progenitor compartment. The binding of erythropoietin to Epo-R prevents apoptosis in the CFU-E pool and the cell cycle progresses into the morphologically recognisable erythroid cell compartment within the bone marrow consisting of pronormoblasts, early normoblasts, intermediate normoblasts, late normoblasts and finally with the extrusion of the nucleus, giving rise to the final stage - reticulocyte.

2. Regulation of Granulopoiesis and Monopoiesis

Granulocytes and monocytes are derived from a common bipotential stem cell (CFU- GM) which is derived from the CFU-GEMM.

1. CFU-GEMM → IL-3 GM-CSF CFU-GM (Colony Forming Unit Granulocyte Macrophage)

2. CFU-GM→ IL-3, GM-CSF CFU-G(Granulocytes) G-CSF

3. CFU-GM IL-3, GM-CSM→ CFU-M (Monocytes/Macrophages) M-CSF

4. CFU-GEMM→ CFU-Eo→ IL-3, GM-CSF, (Eosinophils) IL-5

5. CFU-GEMM→ CFU-Ba→ IL-3 (Basophils)

6. GM-CSF→ Colony Stimulating Factor- Granulocyte, Monocyte/Macrophage

3. Regulation of Lymphopoiesis

The lymphoid stem cell is derived from pluripotential stem cell and gives rise to T and B lymphocytes which are morphologically identical but immunologically and functionally diverse. Lymphopoiesis can be divided into antigen dependent and antigen-independent lymphopoiesis.

Antigen dependent lymphopoiesis occurs in secondary lymphoid organs (adult bone marrow, spleen, lymph nodes, and gut associated lymphoid tissue) and it begins with the antigenic stimulation of the immunocompetent T and B lymphocytes. It results in the formation of effector T and B lymphocytes, which mediate immunity, through the production of lymphokines by T-lymphocytes and antibodies by B-lymphocytes.

Antigen-independent lymphopoiesis occurs in the primary lymphoid tissue (bone marrow, thymus, fetal liver and yolk sac). It begins with the committed lymphoid stem cell and results in the formation of immunocompetent T and B lymphocytes.

4. Regulation of Megakaryopoiesis

Megakaryocytes are derived from pluripotent stem cells, the earliest recognised platelet precursors being the Burst Forming Units (BFU-Meg). Under the influence of thrombopoietin (TPO) and cytokines such as the IL-3 and IL-11, the BFU-Megs develop into megakaryocyte colony forming units (CFU-Meg). Then these mature and develop morphological and biochemical features of megakaryoblasts and megakaryocytes.

The fully mature megakaryocyte has the characteristic platelet granules (alpha granules and dense bodies) and membrane glycoproteins.

STROMAL CELLS

A common mesenchymal precursor cell gives rise to endothelial, fibroblastic and adipogenic marrow stromal cells. This multipotential cell also generates osteoblasts and chondroblasts for bone and cartilage formation. The stroma is composed of fat cells and a meshwork of blood vessels, branching fibroblasts, macrophages, some myelinated and non-myelinated nerve fibers and a small amount of reticulin.

Stromal cells include cells that have been designated reticulum or reticular cells. The marrow fat content varies inversely with the quantity of hemopoeitic tissue. Fat content also increases as bone is lost with increasing age. Bone marrow also contains lymphoid cells, small numbers of plasma cells and mast cells.

CELLULARITY OF THE MARROW

The marrow cellularity is expressed as the ratio of the volume of hemopoeitic cells to the volume of the marrow space. Cellularity varies with the age of the subject and the site. Marrow cellularity is best assessed by histological sections of biopsy or aspirated particles, but should also be estimated from particles that are present in the marrow films.

The myeloid/erythroid ratio is the ratio of total granulocytes to total normoblasts. In newborns and infants, it is somewhat higher than in later childhood or adult life. In adults, the range is broad, varying from 1.2:1 to 4:1. The number of megakaryocytes is estimated more reliably in sections than in marrow films.

BONE MARROW EXAMINATION

This involves examination of two specimens. The first is cytology preparation of bone marrow cells obtained by aspiration of the marrow. The second specimen is needle biopsy of the bone and associated marrow, which allows optimal evaluation of the bone marrow cellularity, fibrosis, infection and infiltrative diseases.

Indications for biopsy:

1. Workup of hematological abnormality observed in peripheral smear

2. Evaluation of primary bone marrow tumors

3. Staging for bone marrow involvement of metastatic tumors

4. Workup for pyrexia of unknown origin

5. Evaluation of metabolic storage diseases

In adults hematopoiesis is limited to the axial skeleton and proximal portion of the extremities. Young children may have a marrow examination done from anterior medial tibial area whereas in adults the sternum or the anterior or posterior superior iliac spine is used.

Bone marrow is semi fluid and easily aspirated through a needle. Most are 14-18 gauge needles and have a removable obturator that prevents plugging of the needle before aspiration, and a stylet that may be used express the bone marrow biopsy sample.

The marrow aspirate is stained with either Wright or Giemsa stain. Accurate evaluation of bone marrow cellularity requires examination of a bone marrow biopsy section. The aspirate smear allows cytologic examination of the bone marrow cells the counts in bone marrow vary according to age also. In general, lymphocytes are more commonly seen in marrow of children, especially in younger than 4 years of age, where they may comprise of up to 40 % of marrow cellularity.

Lymphocytes make up about 20 % of adult marrow cellularity. In adults the erythroid cells make up about 10 to 40 % of marrow cells. The myeloid cells are usually the predominant elements within the bone marrow, and more mature cells are seen. Increased numbers of immature myeloid cells usually indicate a disease process. Megakaryocytes constitute the least abundant cell type in the marrow, making up about 1 % of the cells.

Bone marrow core biopsies are fixed in formalin. The bone marrow biopsy is stained with either hematoxylin or eosin or Giemsa stains for examination. The bone marrow besides providing information about the anatomic distribution and relationship of hematopoietic cells, the bone marrow biopsy is useful for evaluation of focal infiltrative processes such as carcinoma, lymphoma, other tumors, granulomatous inflammation and fibrosis. Occasionally, the marrow is so involved with an infiltrative processes such as carcinoma, lymphoma, other tumors, granulomatous inflammation and fibrosis.

BME is an established diagnostic modality in the evaluation of pancytopenia. BME in most cases gives the specific diagnosis. However, in a few cases, additional tests are required. In the present study, BME was able to establish the diagnosis in most of the cases. Megaloblastic anemia was the commonest finding.

Table 2: HAEMATOLOGY REFERENCE VALUES IN NORMAL ADULTS

TEST	MEN	WOMEN
Hemoglobin	14-17 g/dl	12.3-15.3 g/dl
Hematocrit	41.5 – 50.4%	36 – 45%
Red cell count	4.5 – 5.9 x 10^6 / micro L	4.5 – 5.1 x 10^6 / micro L
White cell count	4.4 – 11.3 x 10^9 / L	4.4 – 11.3 x 10^9 / L
MCV	80 – 96 fl	80 – 96 fl
MCH	27.5 – 33.2 pg	27.5 – 33.2 pg
MCHC	33.4 – 35.5 g/dl	33.4 – 35.5 g/dl
Platelet count	150 – 450 x 10^3 / micro L	l 150 – 450 x 10^3 /micro L
Reticulocyte count	0.5 – 2.5%	0.5 – 2.5 %
ESR	0 – 15 mm / hr	0 – 20 mm / hr

Table 3: DIFFERENTIAL COUNTS OF BONE MARROW ASPIRATE

	OBSERVED RANGE (%)	**MEAN (%)**
NEUTROPHILIC SERIES (TOTAL)	**49.2 – 65**	**53.6**
Myeloblasts	**0.2 – 1.5**	**0.9**
Promyelocyte	**2.1 – 4.1**	**3.3**
Myelocyte	**8.2 – 15.7**	**12.7**
Metamyelocyte	**9.6 – 24.6**	**15.9**
Band	**9.5 – 15.3**	**12.4**
Segmented	**6.0 – 12.0**	**7.4**
EOSINOPHILIC SERIES (TOTAL)		
Myelocyte	**0.2 – 1.3**	**0.8**
Metamyelocyte	**0.4 – 2.2**	**1.2**
Band	0.2 – 2.4	0.9
Segmented	0 – 1.3	0.5
BASOPHILIC AND MAST CELLS	0 – 0.2	< 0.1
ERYTHROCYTIC SERIES (TOTAL)	18.4 – 33.8	25.6
Pronormoblast	0.2 – 1.3	0.6
Basophilic	0.5 – 2.4	1.4
Polychromatophilic	17.9 – 29.2	21.6
Orthochromatic	0.4 – 4.6	2.0
Lymphocyte	11.1 – 23.2	16.2
Plasma cells	0.4 – 3.9	1.3
Monocyte	0 – 0.8	0.3
Megakaryocyte	0 – 0.4	< 0.1
Reticulum cells	0 – 0.9	0.3
Myeloid to erythroid ratio	1.5 – 3.3	2.3

PATHOPHYSIOLOGY OF PANCYTOPENIA

Blood cell production is an enormous and complex process in which a hierarchical developmental progression of primitive, multipotential hematopoietic stem cells gradually lose one or more developmental potentials and ultimately become committed to a single cell lineage, which matures into the corresponding blood cell type. These cells are characterized by the ability to self-renew and differentiate into all mature blood lineages. They are also capable of rescuing lethally irradiated hosts by reconstituting the entire repertoire of hematopoietic cells in the recipients. Hematopoietic stem cells reside predominantly within the bone marrow as quiescent, inactive blood cells, in contact with non-hematopoietic cells that make up the bone marrow microenvironment.

MEGALOBLASTIC ANEMIA

The term megaloblast is a designation that was first applied by Ehrlich to the abnormal erythrocyte precursors found in the bone marrow of patients with pernicious anemia. Megaloblasts are characterized by their large size and by sieve like nuclear chromatin.

A study was carried out over a period of six months at Department of Pathology, Dr RML Hospital in 1999. A total of 250 bone marrow smears were examined. Fifty of these were done in cases of pancytopenia. The commonest cause of pancytopenia was megaloblastic anemia and was seen in 22 out of 50 patients (44%).[8]

A study conducted in the Department of Hematology, Safdarjung Hospital over a period of one year showed 250 cases of pancytopenia of which 200 underwent bone marrow examination. The commonest cause was megaloblastic anemia (72%).[9]

A study done in the Department of Hematology and Transfusion Medicine, Government Medical College and Hospital, Chandigarh over a period of 32 months studied 77 pancytopenia patients. The most common cause was megaloblastic anemia (68%).[5]

A study was conducted in the Medicine Department, Khyber Teaching Hospital, and Peshawar from 1st January 2008 to 30th October 2008 on 50 cases of pancytopenia. Pancytopenia was diagnosed in the presence of anemia (hematocrit value <0.35% in women, <0.40% in men), leukopenia (WBC<3.5x109/L) and thrombocytopenia (platelets <150x109/L). Megaloblastic anemia constituted as the cause in 16% of the total 50 cases.[10]

Bone marrow aspirations were done on patients admitted in a tertiary care center and was studied over a period of 18 months. Pancytopenia was an indication for bone marrow examination in 48 out of a total of 100 cases (48%). Megaloblastic marrow was the cause in 18.75%.[11]

A study done was done in the Department of Pathology, TUTH with 148 cases and of which megaloblastic anemia as cause constituted 23.6%.[3]

Weston CF et al (1987) reported pancytopenia and folate deficiency in three alcoholics. Folate deficiency is a common finding in alcoholics due to abnormalities in diet, intestinal absorption, metabolism and excretion.[12]

Fifty pancytopenia cases were studied in the Department of Pathology, RIMS, Manipur for a period of two years and megaloblastic anemia was found out as the cause in 18%.[13]

Rizwann Aizz Qazzi, Ayesha Massod conducted a study 100 pancytopenia patients collected systematically over a period of 14 months. The most common cause was megaloblastic anemia (28%).[14]

A study done in Hematology-Oncology Department, Yemen on pancytopenia patients for a period of one year during 2005 showed megaloblastic anemia as the cause in 14.7%.[15]

A study done in the Department of Pathology, Medicine in JIPMER showed megaloblastic anemia (38.4%) as the most common cause of macrocytic anemia.[16]

PATHOPHYSIOLOGY OF MEGALOBLASTIC ANEMIA

Megaloblastic anemia is caused by deficiency of folate or cobalamin (Vit B12). The peripheral blood is sometimes characterised by pancytopenia and an increased mean corpuscular volume (MCV). The bone marrow has a prevalence of large, early stage hematopoietic precursor cells. Cytogenetic studies have shown increased chromosomal breakage in the bone marrow. In folate-deficient cells, it is hypothesized that that decreased levels of co-enzyme 5, 10-methylenetetrahydrofolate inhibits the conversion of deoxyuridylate (dump) to thymidylate (dTMP). This results in increased rate of uracil misincorporation into DNA. This mis-incorporated uracil residues that are located near each other on opposing strands of DNA has the potential to produce double stranded DNA breaks. Due to these DNA break, induction of p53, a transcriptional factor with tumor suppressor activity takes place. Along with p53, the protein product p21 also accumulates which results in cell cycle arrest. This cell cycle arrest by p53 has been thought to contribute to its tumor suppressor function. The accumulation of p53 in hematopoietic cells has also been associated with death by apoptosis. So conclusion of this is:

1) Hematopoietic cells undergo apoptosis due to intracellular folate deficiency as well as vitamin B12 deficiency. Since the deficiency of the latter cause's folate trap and folate cannot be utilized, this results in increased uracil mis-incorporation into DNA and increased p53 and p21 proteins.

2) The erythroblasts that survive this apoptotic process give rise to larger than normal reticulocytes (macrocytes).

It is a panmyelosis, affecting all three cell lines even though its name suggests a disorder limited to red cells and erythroid hyperplasia is a prominent feature. The morphological hallmark is nuclear – cytoplasmic dissociation, which is best appreciated in precursor cells in the bone marrow aspirate. Megaloblastic nuclei are larger than normoblastic nuclei and their chromatin appears abnormally dispersed due to its retarded condensation. Random chromosomal abnormalities are seen, including centromere spreading, but non random changes may also occur. Cytoplasmic maturation appears unremarkable.[17, 18, 19]

Macrocytosis of red blood cells is an early change and increases progressively. Individual macrocytes appear first, followed by a gradual rise in MCV that eventually crosses the line (>97fl), long before the hemoglobin level falls.[20] In the case of cobalamine deficiency, with its slow progression, macrocytosis precedes anemia by months.[21]

Eventually poikilocytosis becomes more pronounced with tear drop cells and nucleated red cells, Howell-Jolly bodies and even Cabot rings appear in the blood in severe megaloblastosis.

The functional pathophysiology of megaloblastic anemia is ineffective hematopoiesis in all three hematopoietic cell lines; bone marrow hyperplasia is intense but reticulocytosis does not occur. Many precursor cells are arrested at various stages in interphase but continue to mature. When the process is advanced, most of the precursors die within the hypercellular bone marrow and are phagocytosed. Whether early cell death is primarily apoptotic or not is controversial and may depend upon the model studied.

Peripheral Smear

In megaloblastic anemia, peripheral smear may show pancytopenia. Macroovalocytes, usually with considerable anisopoikilocytosis, is the main feature. Mean corpuscular volume (MCV) is more than 100 fl. In others, MCV may be normal due to excessive fragmentation of red cells. Polychromatophilic cells are reduced. Reticulocyte count may be less than 1%. The leucocyte count is reduced due to reduction in the number of neutrophils and lymphocytes. Hypersegmented neutrophils are usually seen. A minimum of five percent of five lobed or a single six lobed neutrophil is considered significant. Thrombocytes are reduced in number. Macrocytosis and hypersegmented neutrophils occurring together strongly suggests megaloblastic hematopoiesis.[22]

Bone marrow

Megaloblast is a designation first applied by Ehrlich to the abnormal erythrocyte precursors found in the bone marrow of patients with pernicious anemia. These are known to be the morphologic expressions of a biochemical abnormality and retarded DNA synthesis. The aspirate is hypercellular. The M: E ratio is normal or reduced and there is an accumulation of primitive cells due to selective death of more mature forms. The most characteristic finding is dissociation between nuclear and cytoplasmic development in the erythroblasts, with the nucleus maintaining a primitive appearance despite maturation and hemoglobinization of the cytoplasm. Fully hemoglobinized (orthochromatic) erythroblasts, which retain the nuclei, may be seen. The nucleus of the megaloblast has an open, fine and lacy appearance: the cells are larger than normoblasts and an increased number of cells with eccentric lobulated nuclei or nuclear fragments may be present. Mitoses and dying cells are more frequent than normal. Giant and abnormally shaped metamyelocyte and enlarged hyperpolypoid megakaryocytes are characteristic.

APLASTIC ANEMIA

Aplastic anemia is a disease of bone marrow failure characterised by pancytopenia with marrow hypocellularity. It is a disease due to the absence of haematopoiesis, have had parallel histories since the discovery of the function of bone marrow in the mid-19th century.

Neelmann and Bizzozero (1868) observed nucleated erythroid cells in the marrow and concluded that it was the site of continuously proliferating blood cells. Paul Ehrlich (1888) correlated the absence of formed elements in the blood in pregnant women, to severe marrow hypoplasia at autopsy. The disease was named by Vaquez and Aubertin in 1904 "Pernicious anemia with yellow marrow" and emphasized its pathophysiology of failed hematopoiesis which they called anhematopoiesis. Cabot stressed the marrow's distinctive pathology and the need for its examination in the diagnosis. Santesson (1897) recognised toxic substances such as Benzol as a cause of aplastic anemia. In most cases a specific cause usually cannot be elucidated and the disease is labelled as being of idiopathic origin. Daniel et al. (1958) in their analysis of fifty cases of aplastic anemia reported forty three cases of Idiopathic aplastic anemia. The remaining seven cases were attributed due to benzol, phenylbutazone, chloramphenicol and arsenic fruit spray.[23]

Numerous substances added to the list include Sulphonamides (Meyer, Perlmutter, 1942), Mepacrine (Custer, 1946 and Parmer, 1948), Streptomycin (Corelli, 1947) and Tridone. Organic arsenicals, gold compounds and radioactive compounds were reported to cause aplastic anemia. International Agranulocytosis and Aplastic anemia study (1986) confirmed the risk of aplastic anemia with phenylbutazone use and identified even higher probabilities with other NSAIDs.[24]

Aplastic anemia associated with various drugs have been described which include OKT3, ibuprofen and ciprofloxacin.[25]

Aplastic anemia is generally classified as:

1. Idiopathic

2. Secondary to other disorders (acquired aplastic anemia).

3. Constitutional when associated with inherited defects in DNA repair.

A study done in the Department of Medicine and Pathology, PGIMS, Rohtak reported a case of severe aplastic anemia in a previously healthy adult female due to acute parvovirus infection. Laboratory examination showed pancytopenia in peripheral blood and severe hypoplastic bone marrow on biopsy. Serological tests (ELISA) revealed acute parvovirus B infection.[26]

An Indian study has documented the presence of Parvovirus B19 IgM and viral DNA in 40.7% and 37% of aplastic anemia patients respectively, thereby showing an association of parvovirus infection with aplastic anemia.[27]

Rafel M et al. (1998) found transient pancytopenia after Non A, Non B and Non C hepatitis preceding ALL.[28]

Dennis et al. (1978) reported the association of aplastic anemia with Type B viral hepatitis. These patients were positive for HbsAg.[29]

A study done by Gupta showed aplastic anemia as the most common cause constituting 43%.[30]

A total of 111 adult pancytopenia patients aged 13 to 65 years were studied during a one-year period. 45.9% of the pancytopenia patients had a hypocellular marrow and idiopathic aplastic anemia (20.8 %) was the commonest cause.[31]

Aplastic anemia is strongly associated with rare collagen vascular syndrome called Eosinophilic Fasciitis. Pancytopenia with marrow hypoplasia can also occur in Systemic Lupus Erythematosus. This may be due to folate deficiency secondary to hemolysis, infections, treatment with drugs and autoimmunity.

Pereira et al. (1998) have noticed global hypocellularity (47.6%), increased reticulin production (76.2%) and necrosis (19%) in 21 bone marrow specimens from patients with SLE. They concluded that bone marrow might be a target organ in SLE with cytopenias.[32]

Paroxysmal nocturnal hemoglobinuria (PNH) is a clonal disorder arising from a somatic mutation in a hematopoietic stem cell. There is deficiency in complement regulatory membrane proteins such as DAF and CD59 which are covalently attached to the cell membrane by a glycosyl phosphatidyl inositol anchor. Two other syndromes associated with aplastic anemia are Dyskeratosis Congenita characterised by aplastic anemia, reticulated hyperpigmentation, nail dystrophy and mucosal leukoplakia described by Steier N et al.[33] (1972) and Schwachmann-Diamond syndrome associated with pancreatic insufficiency, pancytopenia and hypoplastic marrow described by Schwachmann H et al. (1964).[33]

Fatal pancytopenia in falciparum malaria was reported by Arya et al.31 Namakwa H et al. (1989) have reported a case of plasmodium vivax malaria complicated with pancytopenia due to hypoplasia of the bone marrow.[34]

Plasmodium vivax causing pancytopenia after allogeneic blood stem cell transplantation in a patient with chronic myeloid leukemia was reported by Raina V et al. (1998).[35]

PATHOPHYSIOLOGY OF APLASTIC ANEMIA

Is one of the life threatening disorders among bone marrow failure syndromes. The disorder arises due to specific failure of bone marrow precursor cells or pluripotent stem cells to produce adequate amount of mature hematopoietic cells and hence to fulfil the normal in vivo requirement.[36,37,38,39] In these kinds of disorders the bone marrow precursors are morphologically absolutely normal, might be showing some features of 'marrow stress' along with normal bone marrow stromal distribution.[37,38,39,40] It presents with pancytopenia of variable degree along with hypocellular marrow, in the absence of any specific bone marrow cytogenetics. It may arise as a single cell cytopenia showing failure of one of the committed cell line and later on can lead to marrow aplasia. The hematopoietic cells are replaced by fat cells. The disorder has an appropriate diagnostic criterion. There should be no abnormal cell detected in the peripheral blood or bone marrow, or any evidence of dysplasia.[41]

Major Causes of Aplastic Anemia

• Acquired

Idiopathic

- Acquired stem cell defects
- Immune mediated

Chemical Agents

- Dose related – alkylating agent, antimetabolites
- Benzene
- Chloramphenicol
- Inorganic arsenicals

Idiosyncratic

- Chloramphenicol
- Phenylbutazone

- Organic arsenicals
- Methylphenylethylhydantoin
- Carbamazapine
- Penicillamine
- Gold salts

Physical agents

- Whole body irradiation

Viral infections

- Hepatitis (unknown virus)
- Cytomegalovirus infections
- Epstein –Barr virus infections
- Herpes zoster (Varicella zoster)

• Inherited

- Fanconi anemia
- Telomerase defects

The pathogenesis of aplastic anemia remains unclear, but an autoimmune mechanism appears to be important. The defective function of regulatory T cells leading to increased interferon γ and tumor necrosis factor causing stem cell injury leading to bone marrow aplasia.

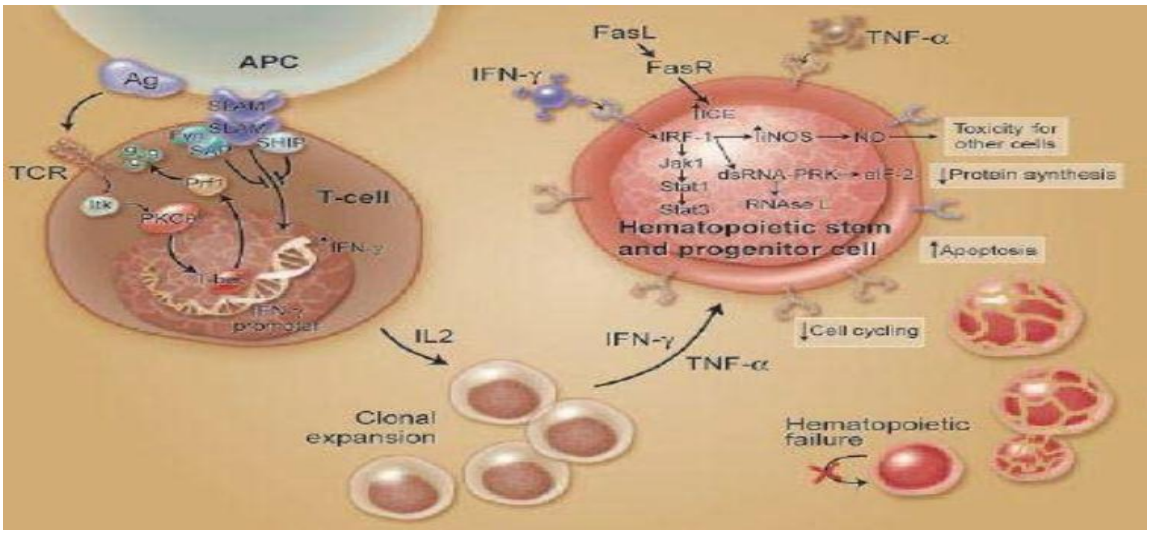


Fig 5: Common pathway leading to stem cell destruction. (**Courtesy:curesearch.org)**

The inciting agents can be in the form of certain antigens presented to T-cells, toxins, drugs, metabolites or viral insults leading to up-regulation of apoptotic genes and expression of Fas-ligand on stem cell surface leading to shortened stem cell survival.

This might be the basis of disease responsiveness to immunosuppressive treatment in many cases. The potential marrow toxins affect certain individuals to produce the disorder and this accounts for genetic predisposition on the high incidence of certain HLA antigens (e.g. HLA DR2) documented in literature.[17, 29]

Peripheral smear

Patients with aplastic anemia have a variable degree of pancytopenia. The hemoglobin percentage is reduced which is usually less than 7 g/dl. Reticulocyte index is low and a retic count of <1% which may be even zero despite high levels of erythropoietin may be seen. Red blood cells show moderate to marked anisocytosis and poikilocytosis.

Anemia is usually normocytic and normochromic but sometimes macrocytic. The leucocyte and platelet counts are low. Neutrophils are more predominantly affected than the lymphocytes. Significant qualitative changes of red cell, leucocyte or platelet morphology are not features of classic acquired aplastic anemia.

Bone marrow

The marrow aspirate typically contains numerous bony spicules with empty fat spaces with relatively few hematopoietic cells, occasionally a dry tap is obtained. Lymphocytes, plasma cells, macrophages and mast cells may be prominent. A marrow biopsy is essential to confirm the overall hypocellularity.

Table 4: DEFINITIONS OF DISEASE SEVERITY OF APLASTIC ANEMIA

Severe AA	BM cellularity <25%, or 25-50% with <30% residual hematopoietic cells Two out of three of the following; • Neutrophils - $<0.5\times10^9$ /l • Platelets - $<20\times10^9$/l • Reticulocytes - $<20\times10^9$/l
Very severe AA	Same as for severe but neutrophils- 0.2×10^9/l
Non- severe AA	Patients not fulfilling the criteria for severe or very severe AA With a hypocellular marrow, with two out of three of the following; • Neutrophils - $<1.5\times10^9$/l • Platelets - $<100\times10^9$/l • Hemoglobin - <10gm/dl

FANCONI's ANEMIA

It is typified by pancytopenia and congenital defects in cutaneous, musculoskeletal and urogenital systems. Pancytopenia may be precipitated by chemical exposure or viral infections. Six of Fanconi anemia genes have been cloned now. FANCA gene is most commonly mutated approximately in 70% of patients.[42]

DYSKERATOSIS CONGENITA

In this inherited disorder the genetic defect affect the telomerase complex, which has both RNA and protein components and usually aplastic anemia develops in the second or third decade.

DRUGS

Chloramphenicol is the most common drug causing aplastic anemia. In vitro chloramphenicol inhibits the growth of both CFU-GM and CFU-E. Amphotericin B is another drug which causes induced myelosuppression and is mediated via release of TNF and INF.[53]

CHEMICALS

Benzene found in organic solvents, coal tar derivatives and petroleum products is a dangerous environmental contaminant. Other aromatic hydrocarbons found in insecticides and herbicides inhibit the Hemopoietic colony formation.[43]

IONIZING RADIATION

Bone marrow cells are affected by both high energy gamma rays as well as adsorbed low energy alpha particles. Bone marrow hypoplasia is observed at total body exposures between 1 and 2.5 Gy. The type and intensity of the radiation source and the distance and shielding of the subject are the major determinants of radiation injury.[42]

VIRUSES

Haematological changes are common in patients with AIDS. It can be nonspecific or specific bone marrow abnormalities, and one of them is bone marrow hypocellularity. Hepatitis associated aplastic anemia causes bone marrow depression when patient is recovering.

Cytomegalovirus infects marrow stromal cells invitro and inhibits their ability to produce growth factors.[42]

PREGNANCY

Bone marrow hypoplasia may be relatively common during pregnancy. Estrogens are related to aplasia of pregnancy and are suggested by the effect of large doses of these hormones on haemopoiesis in animals.[42]

IRON DEFFICIENCY CAUSING PANCYTOPENIA

Iron deficiency anemia is the second most common cause nutritional deficiency in USA. Iron deficiency anemia is usually associated with thrombocytosis but thrombocytopenia associated with Iron deficiency anemia is reported in few cases.[44]

Iron deficiency anemia can be associated with Pancytopenia, though thrombocytopenia has occasionally been reported in Iron deficiency anemia. Although iron deficiency is associated with a reactive thrombocytosis, increasing severity of iron deficiency leads to normalization and occasionally even decrease platelet counts. The exact mechanism of this is unclear but may be related to the alteration in the activity of iron dependent enzymes in thrombopoiesis and leucopoiesis.[45]

The mechanism of leucopenia in unclear, animal experiments and invitro studies using human hematopoietic stem cells have demonstrated that addition of erythropoietin to the stem cells down regulates neutrophil production leading to neutropenia.[45]

PARAOXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)

PNH also called Marchiafava-Micheli syndrome is characterized by the intravascular hemolysis, nocturnal hemoglobinuria, and venous thrombosis and is associated with bone marrow failure.

It is an acquired disease due to nonmalignant clonal expansion of one or several hematopoietic stem cells that have acquired, a somatic mutation of the phosphatidylinositol glycan complementation class A gene (PIG -A).[46]

Intravascular hemolysis is resulting from an intrinsic defect in the membrane of red cells which makes the red cells highly susceptible to complement.

The clinical syndrome can present in 3 types of symptoms including:

1. An acquired intracapsular hemolytic anemia due to abnormal susceptibility of the red cell membranes to the hemolytic activity of the complement.

2. Thrombosis in large vessel, such as hepatic, abdominal, cerebral and subdermal veins.

3. A defect hematopoiesis that may be mild/severe such as pancytopenia in aplastic anemia state.

In 1930's Ham in the USA and Dacie in the UK developed the acidified Ham's test, which became the defining diagnostic test for PNH33. Diagnosis of PNH can usually be established when both the acid hemolysis test and a sucrose hemolysis test are abnormal.

Marcel E.C and James C. Barton studied 43 patients of pancytopenia with bone marrow biopsy specimen containing < 10% nucleated cells. They observed among 43 patients with pancytopenia who had markedly hypoplastic bone marrow specimens, 11 had a positive sucrose test, and four of these had an abnormal quantitative sucrose hemolysis test. They concluded that it's difficult to differentiate PNH from idiopathic AA, both presenting with pancytopenia and thus donor red

blood cell survival studies, which differentiate intravascular from extra vascular hemolytic disorders, permits differentiation of both the disorders.[47]

Pathophysiology Paroxysmal Nocturnal Hemoglobinuria (PNH)

Hemopoietic progenetic number is severely decreased in patients with cytopenias and PNH, even when the marrow is cellular. Both are closely related syndromes. The origin of the sensitive clone is probably an acquired genetic defect in a single enzyme system, responsible for the attachment of phosphoinositol linked proteins to the cell surface, presumably, failure to normally express some cell surface proteins related to growth and proliferation leads to aplastic anemia.

MALIGNANCY

Bloomfield CD et al. (1976) reported haematological parameters of Non-Hodgkin's Lymphoma in one hundred and forty adults. Marrow involvement in poorly differentiated Lymphocytic Lymphoma was associated with pancytopenia in 93% of his patients[48]

Takai K, Sanada M (2000) reported Angioimmunoblastic T cell lymphoma associated with hemophagocytic syndrome.[48, 49]

Pancytopenia was found in 95% of patients with nodular lymphomas in whom marrow involvement was present. Brenner B et al (1985) reported severe pancytopenia associated with angioimmunoblastic lymphadenopathy due to marked marrow fibrosis.[49]

Pancytopenia is present at initial diagnosis in Acute Megakaryoblastic Leukemia. This is due to fibrosis believed to be secondary to the increase in megakaryocytes that secrete platelet mitogenic factor, which stimulates the fibroblasts. This syndrome of „preleukemic aplasia"

appears to be specific feature of common ALL. Pancytopenia can also be seen in Acute Erythroleukemia.

Sultan C described eight patients with acute Myelodysplasia and Myelofibrosis. Four cases were secondary to long term therapy with cytotoxic agents and four were idiopathic.[49, 50]

Harvey M in the clinical review of seventy one cases of hairy cell leukemia found an association with pancytopenia in all the cases.[50]

Zidar B reported seven cases of Hairy cell leukemia, a chronic lympho-proliferative disorder presenting with splenomegaly, pancytopenia and recurrent infection.[51]

Chudgar U et al (1991) studied nine patients of Hairy cell leukemia in five years and found that male to female ratio was 8:1. Weakness and fatigue (66%) was the commonest presenting symptom and splenomegaly (66%) was the commonest physical finding.[49, 51]

Contreras et al (1971) in their study of 4000 bone marrow aspirates and biopsies, observed that bone marrow biopsy is superior to aspiration in the diagnosis of metastatic carcinoma.[3]

Necrosis of bone marrow is a very rare condition with a prevalence ranging from 0.15-7%. Pancytopenia can occur in acute leukemia, lymphomas affecting bone marrow, metastatic tumors, sickle cell disease, tuberculosis, severe bacterial infections and shock. Bone marrow necrosis is best defined as necrosis of myeloid tissue and medullary stroma in large areas of hematopoietic bone marrow. Wade and Stevenson first described bone marrow necrosis.[52]

ACUTE MYELOID LEUKEMIA

Is a disease of elderly and is less commonly seen in childhood. If present, it is usually seen in infancy. It may present in a similar way to ALL, with a period of aplasia, but is much less

common. Rarely, AML may present with a relatively stable degree of aplasia, which may persist for several months before the blood becomes frankly leukemic. In these patients, suspicion may be aroused by finding occasional circulating blasts in the peripheral blood or in a buffy coat preparation and also by the presence of excess blasts in the cells remaining in the bone marrow. Myeloblasts are larger in size compared to lymphoblasts, have fine or open chromatin with prominent or distinct 1-4 nucleoli with moderate amount of cytoplasm. Fragments in the aspirated marrow are usually fleshy and numerous. A bloody tap is not uncommon and occasionally a dry tap occurs. Blasts are the predominant cells comprising 30-95% of the total marrow cells.

Peripheral Smear

Most patients present with pancytopenia and circulating blasts. The WBC count may range from 1000 cells/cumm to 2 lakh cells/ cumm. In 10% of patients who present with modest thrombocytopenia, low grade anemia and normal WBC count without circulating myeloblasts may be seen. A bone marrow examination is required to make the diagnosis. Auer rods in the blasts are virtually pathognomic of acute myeloid leukemia.

Bone marrow

Aspirate is hypercellular with absent or decreased erythroid series and megakaryocytes. However in the elderly, it can be normocellular or hypocellular. Dysplastic myeloid and erythroid maturation may be noted. Myeloid precursors may be morphologically bizarre with asynchronous granulation.

MYELODYSPLASTIC SYNDROME

Myelodysplastic syndromes (MDS) are a heterogeneous group of diseases characterized by active but ineffective hematopoiesis leading to pancytopenia. The term MDS reflects the presence of dysplasia in bone marrow and peripheral blood. Dysplasia may reflect disordered maturation and fragmentation of the nuclear structures, both of which are signs of increased apoptosis.[53]

Characteristic features include:

Occurrence mainly in elderly individuals

• Dysplasia of one or more hematopoietic cell lines with resultant characteristic morphological abnormalities

• Ineffective erythropoiesis due to increased apoptosis, causing cytopenia of one or more cell lines in peripheral blood, and

• Increased risk of transformation to acute myeloid leukemia

• MDS was previously called as dysmyelopoietic syndrome, preleukaemic syndrome, smoldering acute leukemia, and oligoblastic leukemia.

According to the French-American-British (FAB) classification, MDS is said to be present in patients who have less than 30% blasts in bone marrow and peripheral blood and have evidence of ineffective hematopoiesis. Seventeen cases of myelodysplastic syndrome characterised by severe bone marrow fibrosis and a high number of megakaryocytes were studied by Giorgio.[54]

. The most important prognostic factor was the number of blast cells in blood and bone marrow. Seventeen cases of Myelodysplastic syndrome (ten were primary and seven secondary to

previous radiotherapy and chemotherapy), characterised by severe bone marrow fibrosis and a high number of megakaryocytes were studied by Giorgio.[54]

All the patients had pancytopenia and they concluded that MDS with fibrosis might represent a clinicopathological entity, which need to be distinguished from other MDS subtypes as well as from idiopathic myelofibrosis. An International scoring system for evaluating prognosis in MDS was performed using Cytogenetics, morphological, and clinical data by Greenberg P.[55]

Bone marrow fibrosis has rarely been associated with well-defined cases of MDS. An increase in marrow reticulin of varying extent has been reported to occur in 34-50% of primary MDS cases and in 80% cases of MDS secondary to previous anti- neoplastic chemoradiotherapy.[55]

Table 5: BONE MARROW GRADING FOR FIBROSIS

Grade	Bone marrow findings
1+	Represents a fine fiber network with occasional coarse fibers.
2+	A diffuse fiber network with increase in scattered coarse fibers.
3+	a diffuse coarse fiber network with no collagenization (negative trichome stain)
4+	a diffuse network with collagenization(positive trichrome stain)

Thirty one patients of Myelodysplastic syndrome were studied by Kini J and they found Refractory Anaemia with excess blasts in transformation (RAEB-t) to be the commonest subtype (29%) and Refractory anemia with ringed sideroblasts (RARS) the least common (6.4%).[56] Although myelodysplastic syndromes occur predominantly in the elderly, pediatric age group is also affected by MDS.

Presently the WHO Classification-1999 is followed:

- Refractory Anemia
- With ringed sideroblasts
- Without ringed sideroblasts
- Refractory cytopenia with multilineage dysplasia
- Refractory anemia with excess blasts,
- 5q syndrome
- Myelodysplastic syndrome, unclassifiable.

Bennett and Orazi report the conclusions of three workshops held by members of the French–American–British Cooperative Leukemia Working Group. The following approach was used:

1. The presence of unequivocal blasts in the peripheral blood is considered indicative of MDS or AML. Because of significant leukopenia, a precise determination of blast percentage is often impossible, but an effort should be made to count at least 100 cells, including lymphocytes.

2. Hypogranular neutrophils or pseudo-Pelger neutrophils are considered indicative of either MDS or AML if more than 10% are identified. Fewer numbers raise the suspicion but are not felt to be definitive.

3. The presence of >1% to 20% blasts in the marrow aspirates is considered diagnostic of MDS if dysplasia is recognized.

4. Morphological marrow dysplasia of either granulocytes or megakaryocytes is considered as abnormal and inconsistent with AA. Erythroid dysplasia must be moderate to severe, if it is the sole finding (binucleate or trinucleated forms, numerous Howell-Jolly bodies, nuclear budding or bridging).

5. The presence of any abnormal sideroblasts (>5 granules surrounding the nuclear membrane or occupying atleast one third of the circumference) is considered as evidence of dyserythropoeisis and excluded a diagnosis of AA.

6. A 1 to 2 cm core biopsy is preferred for all patients being evaluated for a potential diagnosis of either hypoplastic myeloid disorder or aplastic anemia.

7. The presence of two or more clusters of immature precursors (minimum of three blasts/clusters) in the bone marrow biopsy is indicative of either MDS or AML.

PATHOPHYSIOLOGY OF MYELODYSPLASTIC SYNDROME

MDS are a group of hematological disorders that have a propensity to terminate in acute leukemia. The principal peripheral blood smear findings are pancytopenia, bicytopenia or isolated cytopenias with reticulocytopenia. A macrocytic anemia is common. The bone marrow, however, is normocellular or hypercellular with various degrees of qualitative abnormalities of one or more cell lines (dyshematopoiesis). Signs of dyshematopoiesis are also reflected in morphological abnormalities of one or more cell lineages in the peripheral blood. These qualitative abnormalities of peripheral blood cells are useful in differentiating cytopenias due to true hypoproliferation of stem cells (AA) from cytopenias due to dyshematopoiesis.

Table 6: Morphological abnormalities in MDS

Lineage	Peripheral blood	Bone marrow
1. Erythroid	Oval macrocytes, acanthocytes, elliptocytes, hypochromic fragments, basophilic stippling, nucleated red blood cells.	Ringed sideroblasts, megaloblasts, vacuolated cytoplasm, nuclear fragmentation hyperlobulation, Multinuclearity and inter nuclear bridging.
2. Granulocytic	Nuclear hypolobulation/ hyper segmentation, ringed nuclei, coarse chromatin clumping, cytoplasmic hypo or agranularity	Loss of primary and secondary granules, increased blasts
3. Eosinophilic	Agranular cytoplasm, mixed eosinophilic and basophilic granulation	
4. Monocytic	Abnormal nuclear lobulation, agranular cytoplasm	
5. Megakaryocytic	Agranular or giant platelets	Micromegakaryocytes, large mono or binucleate

Table 7: FAB Classification/ criteria for MDS

Subtype	**Abbreviation**	**Peripheral blood**	**Bone marrow**
Refractory anemia	RA	< 1% blasts	< 5 % blasts
Refractory anemia with ringed sider-oblasts	(RARS)	< 1% blasts	< 5 % blasts, ≥ 15% ringed sideroblasts
Refractory anemia with excess blasts	(RAEB)	< 5 % blasts	Blasts 5-20 %
RAEB in transformation	(RAEBt)	> 5 % blasts	Blasts 20-30 % or Auer rods
Chronic myelomo-nocytic leukemia	CMML	Monocytes > 1000 cells/cumm	Any of the foregoing
Acute myeloid leukemia	AML		Blasts > 30 %

HAIRY CELL LEUKEMIA

Peripheral smear

The recognition of typical hairy cells in peripheral blood films is useful in establishing the diagnosis. Hairy cells are large, twice the size of a normal lymphocyte, and have abundant cytoplasm which is characteristically villous in its outline. The nuclear chromatin is smooth and no nucleoli are seen. These cells are tartrate resistant acid phosphatase positive (TRAP).

Bone marrow

Aspirates are unsuccessful and a trephine biopsy is required. The infiltration is interstitial.

A typical feature is the loose arrangement of the cellular infiltrate, leaving plenty of space between the cells, often with a clear zone around each cell which is unique to this condition.

MULTIPLE MYELOMA

Is a B cell malignancy characterized by a monoclonal expansion and accumulation of abnormal plasma cells in the bone marrow resulting in marrow failure and bony lesions. MM is caused by a clonal proliferation of plasma cells.[57, 58] the following figure: 6 shows bone marrow microenvironment with multiple interactions between myeloma, stromal and endothelial cells and osteoclasts reflecting the vital role of the bone marrow microenvironment in the biology of the disease.

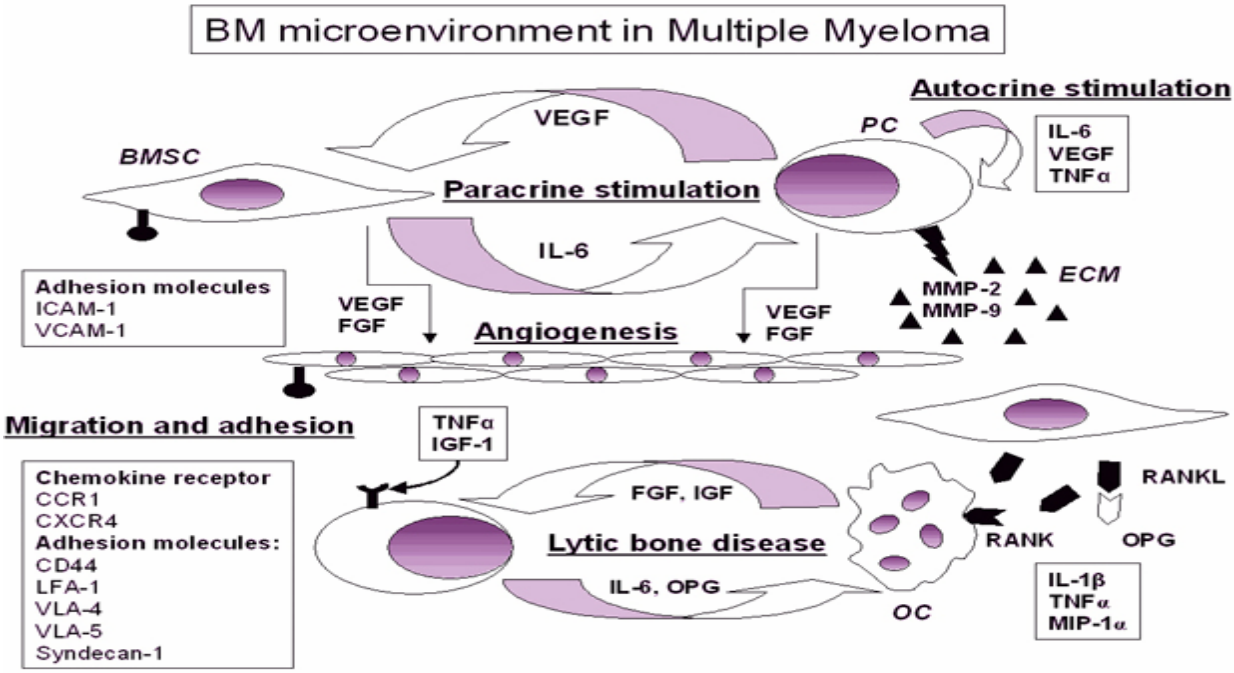


Fig: 6 Bone Marrow Microenvironment in Multiple Myeloma

(Courtesy:curesearch.org)

Peripheral Smear

Anemia may be due to the infiltration of the bone marrow by myeloma cells, due to chronic inflammation or the use of cytotoxic drugs. Thrombocytopenia and leucopenia may also occur due to infiltration of the marrow. Most patients with MM develop anemia of usually moderate severity. Normocytic normochromic blood picture with rouleaux formation and a low reticulocyte count is seen. The macrocytic indices may be the result of red cell agglutination. The ESR is high. The leucocyte count is usually normal or reduced with a mild neutropenia and an increase in plasma cells. The platelet count is usually normal or low.

Bone marrow

Myeloma cells are characteristically present in all the patients. The myeloma cell is moderately large (15-30μm) with a round or ovoid, eccentrically placed nucleus and one or two nucleoli. The bone marrow is infiltrated with an excess of plasma cells (>5%) in approximately 90% of cases.

Criteria for the diagnosis of Multiple Myeloma

Major

• Plasmacytomas on tissue biopsy
• Marrow plasmacytosis with > 30% plasma cells.
• Monoclonal globulin spike on serum electrophoresis (> 3.5g/dl for IgG or > 2g/dl for IgM)

Minor

• Marrow plasmacytosis 10-30%
• Lytic bone lesions
• Normal IgM < 0.05 g/dl, IgA< 0.1g/dl or IgG < 0.6g/dl
• Monoclonal globulin spike is present but is less than the levels defined above.
• Minimum of 1 major + 1 minor or 3 minor criterion are required to diagnose the condition.

MYELOFIBROSIS (MF)

BM fibrosis (Myelofibrosis) refers to the abnormal deposition of reticulin network by bone marrow fibroblasts.Two patterns of fibrosis are seen. Normal pattern is exaggerated leading to reticulin fibrosis and secondarily by bone marrow obliteration by collagen deposition known as collagen fibrosis. Both fibrosis contain type I, III, IV and V with predominance of type III and its precursor.

MF present in 3 stages in the BM and other organs.

1. Cellular phase with pancytosis.
2. MF without marked osteosclerosis
3. Osteosclerosis and hypocellularity with marrow failure.

Table: 8 STAGES OF MYELOFIBROSIS

PREFIBROTIC STAGE	FIBROTIC STAGE
BLOOD	BLOOD
• No or mild leukoerythroblastosis • No or minimal RBC poikilocytosis • Few if any dacrocytes (Tear drop cells)	• Leukoerythroblastosis • Prominent RBC poikilocytosis with dacrocytes (Tear drop cells)
BONE MARROW	BONE MARROW
• Hypercellular • Neutrophilic proliferation • Megakaryocytic proliferation and • atypical (clustering of megakaryocyte, • abnormally lobulated megakaryocytic • Nuclei, naked megakaryocytic nuclei). • Minimal or absent reticulin fibrosis	• Reticulin and/or collagen fibrosis • Decreased cellularity. • Dilated marrow sinuses with intra • luminal haemopoiesis • Prominent megakaryocytic • proliferation and atypical (clustering of megakaryocytes, abnormally lobulated • megakaryocytic nuclei, naked • Megakaryocytic nuclei). • New bone formation (osteosclerosis).

Adverse prognostic indicators in MF are hemoglobin < 10gm %, bone marrow hypocellularity, plasma volume >140% of expected and presence of constitutional symptoms. MF can occur as a secondary phenomenon with many myeloproliferative, malignant and non-malignant disorders and may regress after appropriate therapy directed towards the underlying process.

MF may be reaction to myeloproliferative disorder or to another neoplastic disorder .BM fibroblasts are of mesenchymal origin and not derived from BM stem cells. In primary myelofibrosis, all RBCs, granulocytes and platelets contains the same G-6-PD isoenzymes, suggesting a clonal origin of this disorder .In contrast bone marrow fibroblast contain two different G-6-PD isoenzymes indicating separate origin of fibroblast.[59]

Connective tissue framework of normal bone marrow is synthesized by fibroblasts and consists of a network of reticulin fibers that is continuous with the reticulin of blood vessel wall, sinusoids and endosteum.[59]

PATHOPHYSIOLOGY OF MYELOFIBROSIS

It is a clonal myeloproliferative disorder characterised by the proliferation of mainly megakaryocytic and granulocytic elements in the bone marrow, associated with reactive deposition of bone marrow connective tissue and with extramedullary haemopoiesis.

Peripheral blood Smear

The abnormal replacement of the cells reduces normal hematopoiesis and disrupts the normal bone marrow architecture, allowing release of immature cells into the peripheral blood. Anemia may be accompanied by normal, increased or decreased leucocyte and platelet counts. The most characteristic findings are leucoerythroblastic reaction and a moderate to marked poikilocytosis showing dacrocytes.

Bone Marrow

Bone marrow examination is essential for the diagnosis. There is a stepwise evolution of the disease characterized by prefibrotic and fibrotic stage. Marrow aspiration reveals dry tap, although occasionally normal or even hyperplastic fragments are obtained. Trephine biopsy is a reliable diagnostic procedure.

Smears from successful aspirates may show no abnormality, but usually there is neutrophilic and megakaryocytic hyperplasia. The megakaryocytes are often morphologically abnormal. Micromegakaryocytes and macromegakaryocytes are often observed.

Erythroid precursors may be normal or increased. Granulocytes may show hyper or hypolobulation, acquired Pelger-Huet anomaly, and nucleo-cytoplasmic asynchrony.

Bone marrow biopsy is necessary to demonstrate fibrosis. Histologic evidence of osteosclerosis may be present. Bone marrow sinusoids are expanded, and there is intravascular haemopoiesis. Increased number of mast cells may be observed in biopsy adjacent to fibrosis.

As the disease evolves, haemopoiesis frequently becomes ineffective and blood cell counts fall leading to pancytopenia. Products of cells are released in the marrow, including the platelet derived growth factor from megakaryocytes and stimulate deposition of reticulin and fibrous tissue.

INFECTIONS CAUSING PANCYTOPENIA

Bacterial, fungal, protozoal and viral infectious agents are sometimes associated with pancytopenia, which ranges from mild depression in blood counts to severe trilineage aplasia. It may be caused by direct toxic effects leading to bone marrow necrosis, bone marrow replacement (myelophthisis), inhibitory effects of inflammatory mediators, or activation of the reticuloendothelial system leading to hemophagocytic syndrome. Persistent congestive splenomegaly as a result of infection can also cause pancytopenia.

Viral infections associated with pancytopenia include those with arboviruses such as dengue, which cause transient effects, and suspected agents such as Non A to Non C hepatitis which appear to be associated with more durable aplastic anemia.

Blood Anemia is usually normocytic, normochromic or mildly microcytic and hypochromic with a low reticulocyte count. Changes in leucocyte parameters include cellular morphology and number. Early changes in infection include an increase in band forms and a greater shift to immaturity (left shift) which occurs in severe infections. Toxic granulations, cytoplasmic vacuolization and Dohle bodies are seen.

BRUCELLOSIS

Brucellosis is a zoonotic infection existing worldwide, with predominance in central Asia and some developing countries. Brucellosis is caused by small, fastidious gram negative coccobacilli of the genus brucella. B.melitensis is the most invasive and causes the most severe disease. Bone marrow and spleen are commonly involved and such involvement may result in a hypoplastic pattern on the peripheral blood smear.pancytopenia in Brucellosis may be seen due to hemophagocytosis, hypersplenism, bone marrow granulomas, bone marrow hypoplasia and immune destruction.

Sari Ismail studied 202 cases of brucellosis out of which 30 patients had pancytopenia and concluded as histiocytic hemophagocytosis was a major cause of pancytopenia in the patients with brucellosis.[60]

EHRLICHIOSIS

Ehrlichia, members of the family Rickettsiaceae are obligate intracellular bacteria that parasitized circulating mononuclear or neutrophils.

Since 1987 over 150 cases of human ehrlichosis have been reported. Most of the patients have nonspecific febrile illnesses with myalgia, headache, gastrointestinal symptoms, relative

bradycardia and cytopenia .Bone marrow examination disclosed a variety of abnormalities including marrow hypoplasia mainly.[61]

TUBERCULOSIS

Tuberculosis exerts a dazzling variety of hematologic effects. It is postulated that the release of TNF-alpha and other cytokines by TB – activated monocytes suppress the erythropoietin production that normally occurs in the setting of anemia of chronic disease.

The various postulated mechanism for pancytopenia include splenic sequestration and immune mediated bone marrow suppression. Decrease bone marrow reserve is also due to malnutrition.

Pancytopenia is a consequence of the combined effects of hypersplenism, excessive margination of neutrophils and or marrow granulopoietic failure mediated by the expansion of T-lymphocytes showing granulopoietic inhibitory activity.[62]

MALARIA

It was the Italians in the 18th century who named the desire malaria meaning "foul air".

Malaria is a parasitic infection caused by obligate intracellular protozoa of the genus plasmodium, four species causing human disease are Falciparum, Vivax, P.ovale and P.malarie.

Hemolytic anemia due to the destruction of erythrocytes by parasite can manifest as pallor, fatigue and hemodynamic derangement. Thrombocytopenia is a common manifestation and is immune related and in severe infection is due to disseminated intravascular coagulation.

Malaria due to Falciparum infection has the distinction of being the most devastating of the malarial illness. Severe disease with multiorgan dysfunction is seen only in Falciparum malaria and is due to the ability of the organism to achieve heavy parasite burden and the intravascular

sequestration of parasitized erythrocytes leading to impaired oxygen delivery and subsequent end organ damage.

Plasmodium falciparum parasites usually are found in erythrocytes of normal size. In Plasmodium falciparum infection, ring forms predominate and finding numerous ring forms without mature stages is evidence for plasmodium falciparum infection, young rings being smaller. The presence of doubly infected cells and double chromatin dots in ring trophozoites occur more commonly in plasmodium falciparum. Gametocytes of plasmodium falciparum are readily identified by their characteristic sausage shape.

An inadequate bone marrow response to anemia is seen, with relative reticulocytopenia. Leucocyte number may be slightly increased or normal, but leucopenia as a result of splenomegaly and impaired marrow function is characteristic.

Thrombocytopenia is seen in nearly 70% of infections. The bone marrow reactions caused by plasmodium vivax are qualitatively similar to those caused by plasmodium falciparum not only in the red cell lineage but also in other cell lines, characterized by dyserythropoeisis and ineffective erythropoiesis.[63]

Peripheral Blood

Anemia is usually normocytic, normochromic or mildly microcytic and hypochromic with a low reticulocyte count. Trophozoites and schizoints of malarial parasite may also be seen. Changes in leucocyte parameters include increased leucocyte count and increase in the number of monocytes. Thrombocytopenia is a common finding, the mechanism being suppression of platelet production, increased platelet utilization.

Bone Marrow

The marrow is often hypocellular. Granulopoiesis and erythropoiesis in particular may be markedly decreased. The number of megakaryocytes in the marrow is normal or reduced slightly. Malarial parasite can be seen.

HIV

Hematological abnormalities are well recognised in HIV diseases and result from diverse influences on hematopoietic tissue changes in the peripheral blood and bone marrow which may reflect disease elsewhere in the body and resist the treatment for that disease or may reflect an attempt to attack the virus itself or may seem to be an isolated hematological disorder.

In virtually all patients with advanced AIDS pancytopenia is the rule. The causes are heterogeneous and commonly iatrogenic or multifactorial. The most consistent hematopoietic defects that occur in the seropositive patients as a result ofHIV-1 infection, Per se, includes regenerative bone marrow failure in which on demand hematopoiesis is suppressed.

The occurrence of antibodies against blood cells in patients with HIV infection can be due to

• Production of the antibodies which might be triggered by the exposure of crypt antigens as a consequence of infection related damage of blood cells, especially platelets and granulocytes.

• The hematopoietic cells especially platelets and granulocytes are antigenically similar to agents like HIV and other microorganisms infecting the patients. These antibodies could interact with tissue antigens.

• A third possibility is that HIV acts as the direct inducer of autoimmunity.

Haematological abnormalities are among the most common complication of HIV. These involve all lineages of blood cells. These complications seem to be dependent on the level of viral replication and viral load. Anemia is most common complication and its incidence is strongly associated with the progression of the disease. Neutropenia is common in advanced stage of disease and thrombocytopenia is co-related with low CD4+ cell count and older age.

A known complication of HIV infection in the bone marrow is dyspoietic hematopoiesis termed as HIV myelopathy. HIV myelopathy, unlike a true MDS is not considered a true stem cell disorder but rather represents a spectrum of morphological changes secondary to direct HIV effect and HAART.[64]

HYPERSPLENISM

Green D et al. (1971) presented a case of Gauchers disease with hypersplenism and pancytopenia.[65]

Pancytopenia is one of the well-known hematologic manifestations of hypersplenism. Banti (1800) and Gristel (1866) explained the idea that spleen may produce ill effects through exaggeration of its normal activities. Chaufford (1907) introduced the term hypersplenism to refer to this concept.

Johnson HA et al. (1989) in a review of 391 splenectomies performed over a sixteen year period, observed that pancytopenia and hemolytic complications of the disease processes were the commonest indications for splenectomy.[65]

Cruck and Riser (1949) have suggested the following criteria for the diagnosis of hypersplenism:

1. A massively enlarged spleen.

2. Depleted cell values including anemia, neutropenia and thrombocytopenia occurring either singly or in combination.

3. Bone marrow hyperplasia with regard to the cells, which are deficient in the blood.

4. Demonstration of increase in cell values after splenectomy.

PATHOPHYSIOLOGY OF HYPERSPLENISM

Hypersplenism can occur as a primary event due to an unknown pathogenic stimulus.

Some of the important causes of secondary hypersplenism are haematological malignancies, storage disease, infections like malaria, typhoid, brucellosis, leishmaniasis, collagen vascular diseases, congestive splenomegaly and splenic tumors.

The pathogenesis of hypersplenism is explained as follows.

Anemia: Sequestration and hemodilution combine to produce the anemia of hypersplenism. An expansion of the plasma volume accompanies hypersplenism and the degree of expansion is proportional to the size of the spleen.

Neutropenia: The neutropenia of hypersplenism is caused by an increase in the marginated granulocyte pool, which is located in the spleen.

Thrombocytopenia: Increased splenic platelet pooling. A massively enlarged spleen can hold ninety percent of the total platelet mass.

Hypersplenism is a clinical syndrome; it does not imply a specific causal mechanism. It has the following characteristic features.

1) Enlargement of spleen.

2) Reduction in one or more of the cell lines in the peripheral blood.

3) Normal or hyperplastic cellularity of the bone marrow, often with orderly maturation of earlier stages but paucity of more mature cells.

4) Premature release of cells in the peripheral blood, resulting in reticulocytosis and/or large immature platelets.

5) Increased splenic red cell pool, decreased red cell survival and increased splenic pooling of platelets with shortening of their life span.

Peripheral blood – Anemia is usually normocytic normochromic. Marked anisopoikilocytosis is rare. The total WBC count ranges from 1000-4000 cells/cumm. A moderate thrombocytopenia is usual.

Bone marrow – The picture is of either normal cellularity or hypercellularity. Erythropoiesis is usually normoblastic. The disease process which has been responsible for the enlargement of spleen may infiltrate into the marrow. In some cases white cell precursors show an arrest at the myelocyte or metamyelocyte stage. The megakaryocytic cells may also show maturation arrest.

OTHER INFECTIONS

Bacterial, fungal, viral and protozoal agents are sometimes associated with pancytopenia, which ranges from mild depression in blood counts to severe trilineage aplasia. It may be caused by direct toxic effects leading to bone marrow necrosis, bone marrow replacement (myelophthisis), inhibitory effects of inflammatory mediators, or activation of the reticuloendothelial system leading to hemophagocytic syndrome. Persistent congestive splenomegaly as a result of infection can also cause pancytopenia.

Viral infections associated with pancytopenia include those with arboviruses such as dengue, which cause transient effects, and suspected agents such as Non A to Non C hepatitis that appears to be associated with more durable aplastic anemia.

Peripheral Smear

Anemia is usually normocytic normochromic or mildly microcytic and hypochromic with a low reticulocyte count. Early changes in infection include an increase in band forms and a greater shift to left. Toxic granules, cytoplasmic vacuolations and dohle bodies are seen. Thrombocytopenia in the absence of disseminated intravascular coagulation (DIC) may be seen in septicemia associated with bacterial, fungal or viral infections. Viral infections like dengue shows atypical lymphocytes.

Bone marrow

The marrow is often hypocellular. Granulopoiesis and erythropoiesis may be markedly decreased. The number of megakaryocytes in the marrow may be normal or slightly reduced.

OTHER CONDITIONS CAUSING PANCYTOPENIA

PURE RED CELL APLASIA (PRCA)

In adults this is acquired, with profound anaemia, characteristically without reticulocytes in the blood and erythroid precursors absent from the marrow while the other elements are preserved.

Pathophysiology of PRCA involves total loss of erythropoiesis is believed to be immune-mediated by both antibody and T-cells at a point of development prior to the pro-erythroblasts. Additionally there may be humourly directed inhibition of erythropoietin but the alternative explanation is that erythropoietin stimulating activity leads to the loss of these precursors. Many cases have shown specific attack on the precursors by parvovirus B19 and increasing concern of an underlying early myelodysplasia. The etiology may be primary or secondary. Clinical

Examination shows severe degrees of anaemia that is, initially at least, asymptomatic but typically preceded by fall in exercise tolerance and relentlessly progressive fatigue.

Peripheral blood smear and bone marrow

Reticulocytes are absent but the remainder of the peripheral blood findings are normal. Erythroblasts are reduced or absent, white blood cell and platelet production is normal.

HEREDITARY ELLIPTOCYTOSIS (HE)

Hereditary elliptocytosis (HE) is a congenital hemolytic disorder characterized by an elongated (cigar or oval) shape of erythrocytes.[78] This disorder is the result of a defect in one of the cytoskeletal proteins in the red blood cells membrane that are usually responsible for the elasticity and durability of circulating erythrocytes. A few mutations of the alpha-spectrin subunit are responsible for most cases of HE.

HE also occurs with deficiencies in protein 4.1 or glycophorin C or when defects of band 3 protein or beta-spectrin subunit impair ankyrin binding .[66] Although spontaneous mutations have been reported, HE is transmitted predominantly as an autosomal dominant trait with at least 4 genetic loci implicated.[67]

Most patients are asymptomatic and present with intermittent episodes of more intense hemolysis with anemia, jaundice, and splenomegaly .Typical presenting signs in patients with severe HE include neonatal hyperbilirubinemia and anemia in the first few months of life. In complications of severe hemolysis, including anemia, splenomegaly, and growth retardation, frontal bossing, and early gallbladder disease .Members of the same family may exhibit different clinical courses, and an individual's frequency and severity of hemolysis may change with time.

Peripheral blood smear and bone marrow

The hallmark of HE is the presence of cigar-shaped elliptocytes on the peripheral blood smear. Elliptocytes are normochromic and normocytic and range from few to 100% of the erythrocytes. Spherocytes, ovalocytes, stomatocytes, microcytes and fragmented cells may also be observed. The elliptical erythrocyte form is acquired in the circulation and that is why the reticulocytes and bone marrow red blood precursors are normal in shape.

CLINICAL FEATURES OF PANCYTOPENIA

The onset of the disease is insidious, manifestations depend on severity of anemia, leucopenia, and thrombocytopenia.

Initial presenting symptoms include mild progressive weakness and fatigue attributable to anemia. Also patients are predisposed to various infections because of neutropenia.

Hemorrhage from skin, nose, and gums is due to thrombocytopenia.

Physical examination reveals fever, pallor, petechial and ecchymosis patches over the skin, mucous membranes, and conjunctiva. Presence of splenomegaly and lymphadenopathy calls attention to the possibility of leukemia, lymphomas, and myelofibrosis and storage diseases.

On the other hand, lack of these signs and absence of evidence of vitamin B12 or folate deficiency should suggest multiple myeloma or aplastic anemia. Finally rare presentations include diarrhea, jaundice and weight loss.

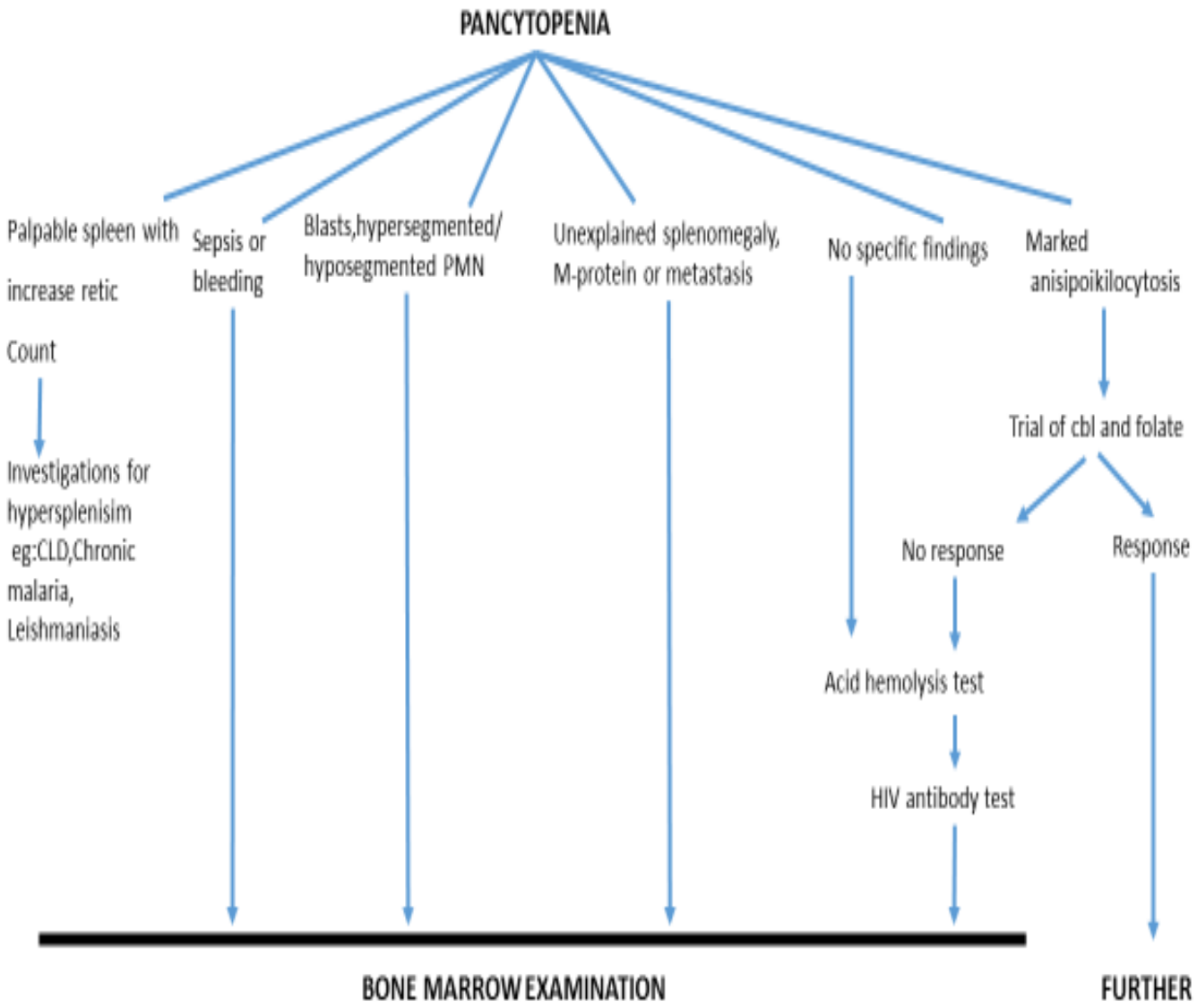


Fig: 7 Shows the diagnostic approach to pancytopenia

METHODOLOGY

Sri Devaraj Urs Medical College and R L Jalappa Hospital and Research Centre is a tertiary care hospital and daily around 150-200 complete hemograms are done in the hematology department which includes both inpatients and outpatients.

Patients diagnosed to have pancytopenia based on hematological criteria and requiring bone marrow examination was studied at R L Jalappa Hospital and Research Centre. Seventy patients were selected who have pancytopenia on peripheral blood smear based on hematological criteria of hemoglobin less than or equal to10 gm /dl, Total leucocyte count less than or equal to 4000/cumm, Platelets less than or equal to 1, 00,000/cumm were selected. Patients on myelotoxic chemotherapy were excluded from the study.

The study included analysis of 70 prospective cases in Central laboratory of R L Jalappa Hospital and Research Centre was studied from 2013 to 2015. Hence it is a prospective study.

Patient details such as age, sex, drug history, occupation, past medical illness, general examination of all the identified cases of pancytopenia were done as per the proforma.

2 ml of EDTA(Ethylene-diamine-tetra-acetic acid) anti-coagulated blood was collected and processed through a hematology analyzer and parameters like complete blood cell count ,total leucocyte count, differential count, Mean corpuscular volume(MCV), Mean corpuscular hemoglobin concentration (MCHC), Mean corpuscular hemoglobin (MCH) Packed cell volume (PCV) were evaluated by Beckman Coulter based on the hematological criteria. Internal Quality control is run every day in hematology department. Corrective measures are taken if values go

beyond ± 2 SD. External Quality control is also performed as our hematology department registered with All India institute of medical sciences, New Delhi.

Peripheral smear was studied by Leishman stain for morphological detail.

Out of seventy patients, bone marrow aspiration was performed in 67 cases, three cases where the aspiration was unsuccessful biopsy was performed.

After taking informed consent from the patient or the guardian bone marrow aspiration or biopsy was done on posterior iliac crest of the patients under aseptic precautions.

Bone marrow smears were made and stained with May-Grünwald Geimsa (MGG) Stain.

STAINING PROCEDURE

Logistics and materials:

1. Leishman stain
2. Buffered distilled water (pH 6.8-7.2)
3. Timer
4. Slide
5. EDTA blood sample

Smear preparation

1. Smear was covered with Leishman's stain
2. It was allowed to stand for 1-2 minutes.
3. Without removing the stain, double the amount of buffered distilled water was added.
4. Allowed it to stand for 7 minutes.
5. Slide was flooded with tap water.
6. Back of the slide was washed with soap and water
7. It was air dried in a tilted / upright position

A Well stained film had the following features:

- The nuclei of leucocytes was purple
- Neutrophilic granules – tan in colour
- Eosinophilic granules – red orange in colour
- Basophil – dark purple granules
- Platelets – had dark lilac granules
- Cytoplasm of lymphocytes – light blue
- RBC's – pink colour

Bone Marrow Aspiration

This was done in sixty seven patients under proper sterile precautions. First an informed consent was obtained. Then the patient was positioned properly and local anaesthesia was given after a test dose. A Salah's needle was used to aspirate material from right posterior iliac crest in majority of cases.Figure:8a, 8b The needle and the stillette were placed in position and the cap was closed. After piercing the skin and the subcutaneous tissue, the periosteum and cortex were pierced in a clockwise rotatory motion. Once in the marrow cavity, the stillette was removed and 0.2-0.3 ml of marrow fluid was aspirated with a sterile disposable 20ml syringe. The aspirate was then transferred to a watch glass filled with 5ml of sodium citrate and was mixed gently. Then with the help of a needle, marrow particles were transferred to the slides and smears were made and allowed to dry. Slides were then stained with Leishman's stain and MGG. The bone marrow aspirate smear is a preparation designed to spread the cellular material of the marrow so that Romanowsky's stain can reveal essential cellular details.

STAINING PROCEDURE:

1. May Grunwald Geimsa(MGG)
2. Buffered distilled water (pH 6.8-7.2)
3. Slide
4. Bone marrow aspirate sample

Smear preparation

1. Smear was covered with Geimsa(MGG) stain
2. It was allowed to stand for 1 minute.
3. Without removing the stain, double the amount of buffered distilled water was added.
4. Allowed it to stand for 15-20 minutes.
5. Slide was flooded with tap water.
6. It was air dried in a tilted / upright position

The bone marrow aspirate was evaluated as follows:

1. A low power scan of the bone narrow aspirate was done to see if the material obtained was satisfactory and also to assess its cellularity.

2. A high power view and oil immersion lens were used to determine the distribution and morphology of cell types.

The smears were assessed as per the following format:

i. Cellularity of the fragments

ii. Erythropoiesis - cellularity, maturation pattern and any cytological abnormalities

iii. Myelopoiesis - cellularity, maturation pattern and any abnormalities

iv. M: E ratio – to count 500 cells

v. Megakaryopoiesis – number, morphology, presence of immature forms

vi. Lymphocytes

vii. Plasma cells

viii. Parasites / abnormal cells / Granulomas / storage cells

Bone Marrow Biopsy

This was done in three cases where aspiration was unsuccessful. Following aspiration, another needle (Trephine) was pushed into the cavity at the same site and the stillette was removed and

then pushed further into the cavity by clockwise rotating movement for about 1-1.5 cm. This procedure captures the marrow core sample within the needle. The needle was then withdrawn in anticlockwise rotating motion. Then the stillette was inserted and biopsy taken out. Imprints were taken on a glass slide. The specimen was fixed in 10% formalin overnight and decalcified with EDTA. Then it was processed similar to histopathological sample and H & E sections were studied.

The microscopic examination of fixed, embedded sections of the bone marrow tissue as represented by biopsy is best for determining the overall cellularity and presence of infiltrates. A much larger percentage of the bone marrow can be scanned in the biopsy specimens compared to the bone marrow aspirate. Touch imprint preparations from the biopsy material are also useful for morphology.

Trephine Biopsy Interpretation - H & E sections were studied as follows:

Adequacy of the biopsy – the ideal specimen was 1-2 cm in length, and was not distorted and had at least 5 well preserved intertrabecular marrow spaces for interpretation.

i. Cellularity - Normocellular/ hypocellular/ hypercellular
ii. Erythropoiesis - cellularity and distribution
iii. Myelopoiesis - cellularity and distribution
iv. Megakaryopoiesis - number, morphology, immature forms
v. Marrow fibrosis
vi. Presence of abnormal cells / parasites / granulomas
vii. Miscellaneous – amyloidosis, necrosis, gelatinous transformations etc.

STATISTICAL ANALYSIS

Frequencies: The frequencies procedure provides statistics and graphical display that are useful for describing many types of variables. For a frequency report and bar chart, we can arrange the distinct values in ascending or descending order or order the categories by their frequencies. The frequencies report can be suppressed when a variable has many distinct values. We can label charts with frequencies or percentages.

Chi square test: Continuous data was summarized in percentage. The categorical variables were compared by chi-square test. Pearson's correlation analysis was used to asses association between the variables. A p value <0.05 was considered statistically significant. All analyses were performed on SPSS Software (22 version).

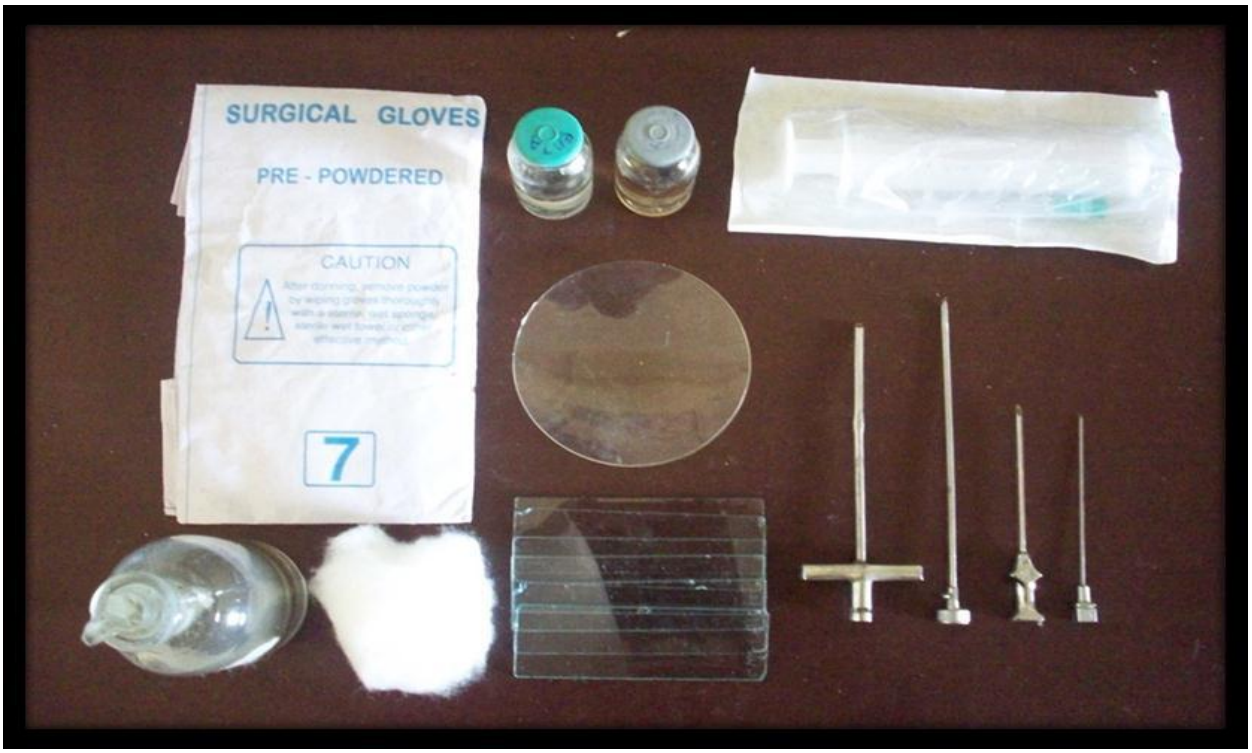


Fig: 8a Materials Used for Bone marrow aspiration and biopsy

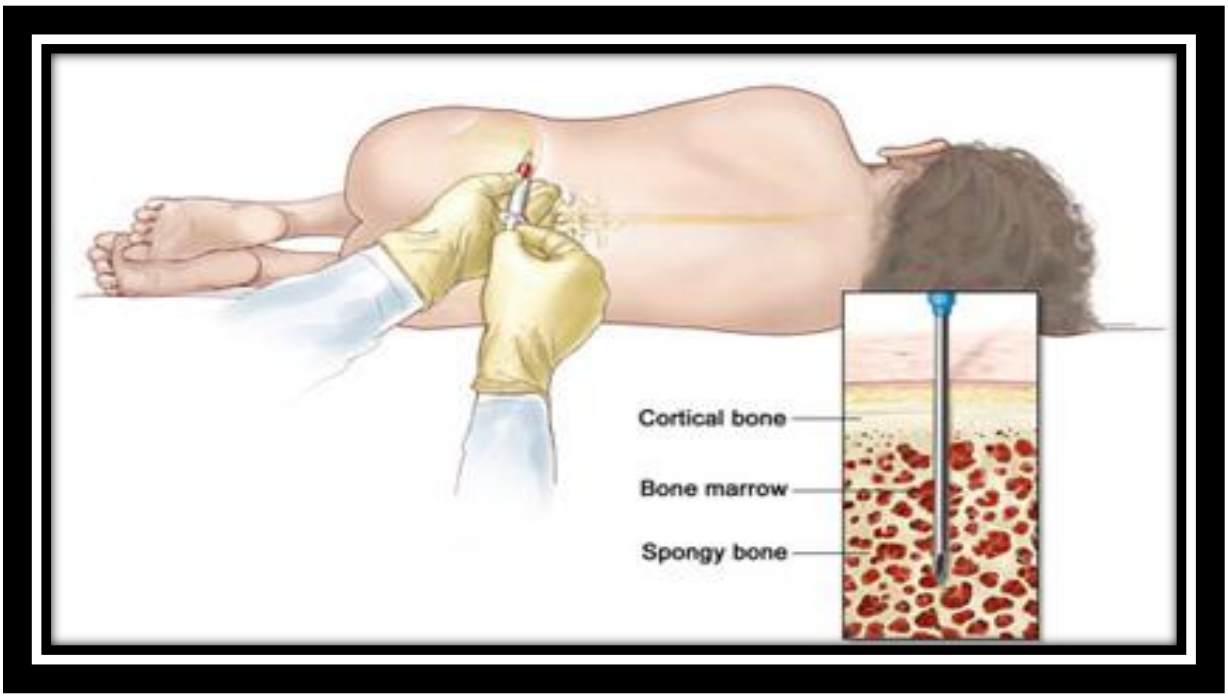


Fig: 8b Shows bone marrow aspiration site and procedure

(Courtesy:curesearch.org)

RESULTS

Seventy patients with a hematological diagnosis of pancytopenia were studied during the period December 2013 to December 2015 in the Department of Pathology, Sri Devaraj Urs Medical College, Tamaka, Kolar.

Out of seventy patients, bone marrow aspiration was done in 67 cases, in 3 cases where aspiration was unsuccessful biopsy was confirmed. The following data were recorded and analyzed. Primary haematological procedures were carried out in all 70 patients, in which haematological parameters and peripheral smears and bone marrow were examined in detail.

The following parameters are discussed below:

- Age distribution and Sex distribution
- Presenting symptoms and signs
- Hematology data – Hemoglobin, Leucocyte count, platelet count, MCV.
- Peripheral blood smear findings
- Bone marrow cellularity
- Erythroid series findings
- Myeloid series findings
- Megakaryocyte findings

Table: 9 Age and sex distribution in cases of pancytopenia

AGE(in years)	GENDER		TOTAL	PERCENTAGE (in %)
	MALE	FEMALE		
<20	3	6	9	12.9
20-30	5	9	14	20
31-40	5	7	12	17.1
41-50	3	6	9	12.9
51-60	12	4	16	22.9
>60	3	7	10	14.3
Total	31	39	70	100

As shown in the table:9 and figure:9, most of the patients were in the age group of 51-60yrs seen in 16 (23.9%) and the least occurrence was seen in the age group of below 20yrs and 41- 50 years (13%). The sex distribution of pancytopenia showed a female preponderance. The female to male ratio was 1.25:1.

Fig: 9 Age and sex distribution in pancytopenia

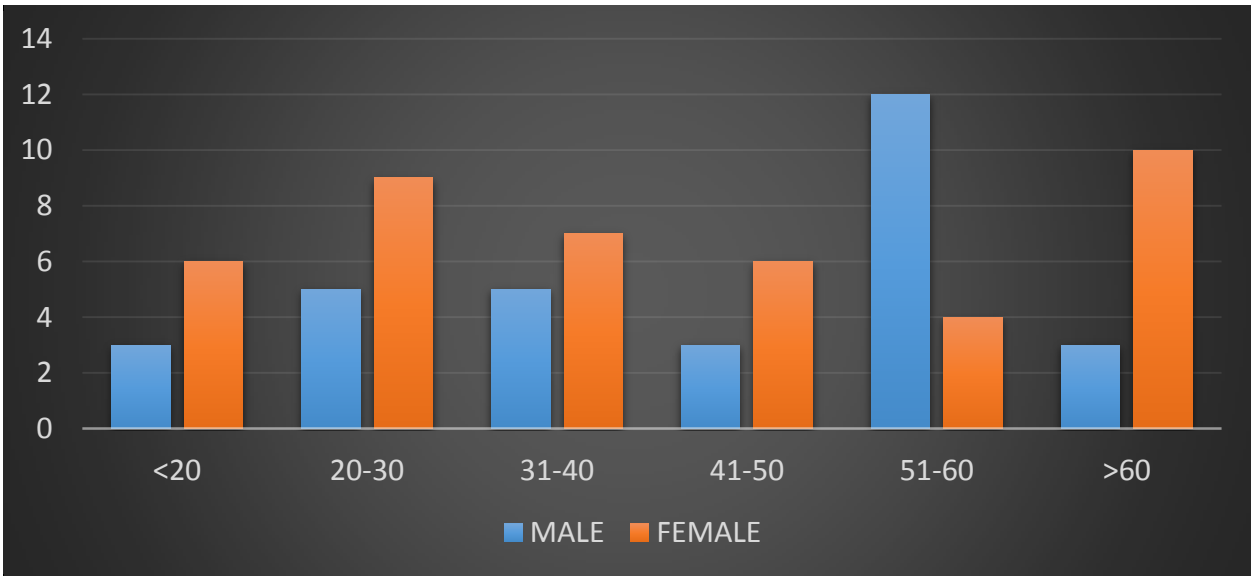


Table: 10 Distribution of presenting symptoms in pancytopenia patients

SYMPTOMS	TOTAL	PERCENTAGE (in %)
Generalised weakness	37	52.8
Fever	47	67.1
Easy fatigubility	33	47.1
Pain abdomen	8	11.4
Breathlessness	2	2.8
Altered sensorium	1	1.4
BLEEDING MANIFESTATIONS	7	10
1. Petechial hemorrhage 2. Gum bleeding 3. Hemoptysis 4. Vomiting/hematemesis 5. Nasal bleed 6. Blood in stools	2 1 1 1 1 1	

As shown in the table: 10 and figure: 10 fever 47(67.1%) was the most common symptom and the least common symptom was breathlessness2 (2.8%) and altered sensorium1 (1.4%).

Fig: 10 Distribution of presenting symptoms in pancytopenia patients

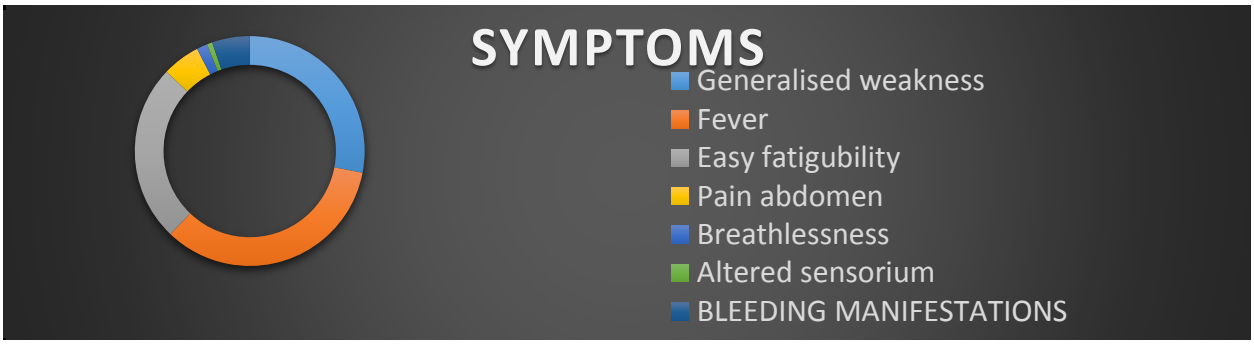


Table: 11 Range of hematological parameters in pancytopenia

HEMOGLOBIN (in gm/dl)	NUMBEROF PATIENTS	PERCENTAGE (in %)
1-3	10	14.9
3.1-5	24	34.2
5.1-7	14	20
7.1-10	22	31.4
TOTAL	70	100
LEUKOCYTE COUNT (cells/cumm)	**NUMBER OF CASES**	**PERCENTAGE (in %)**
500-1000	3	4.2
1100-2000	9	12.8
2100-3000	21	30
3100-4000	37	55.2
TOTAL	70	100
PLATELET COUNT (cells/cumm)	**NUMBER OF CASES**	**PERCENTAGE (in %)**
4000 - 25000	17	24.2
26000 - 50000	17	24.2
51000 - 75000	13	18.7
76000 -100000	23	34.3
TOTAL	70	100
MCV (fl)	**NUMBER OF CASES**	**PERCENTAGE (in %)**
51 - 70	7	10.4
71 - 90	33	47.2
91 - 100	15	22.3
101 -110	10	14.2
111 - 120	5	7.4
TOTAL	70	100

As shown in the table:11 and figure:11(34.2%) showed maximum hemoglobin range of 3.1-5 gm/dl and minimum hemoglobin range was 1-3 gm/dl seen in 10(14.9%) of the cases. 37(55.2%) of the cases showed maximum Leucocyte range of 3100-4000/cumm and minimum leucocyte range was 500-1000/cumm seen in 3(4.2%) of the cases.23(34.3%) cases showed maximum platelet count range of 76,000-1,00,00 and minimum platelet count range was seen in 10(14.9%) of the cases

Fig: 11 Range of hematological parameters in pancytopenia

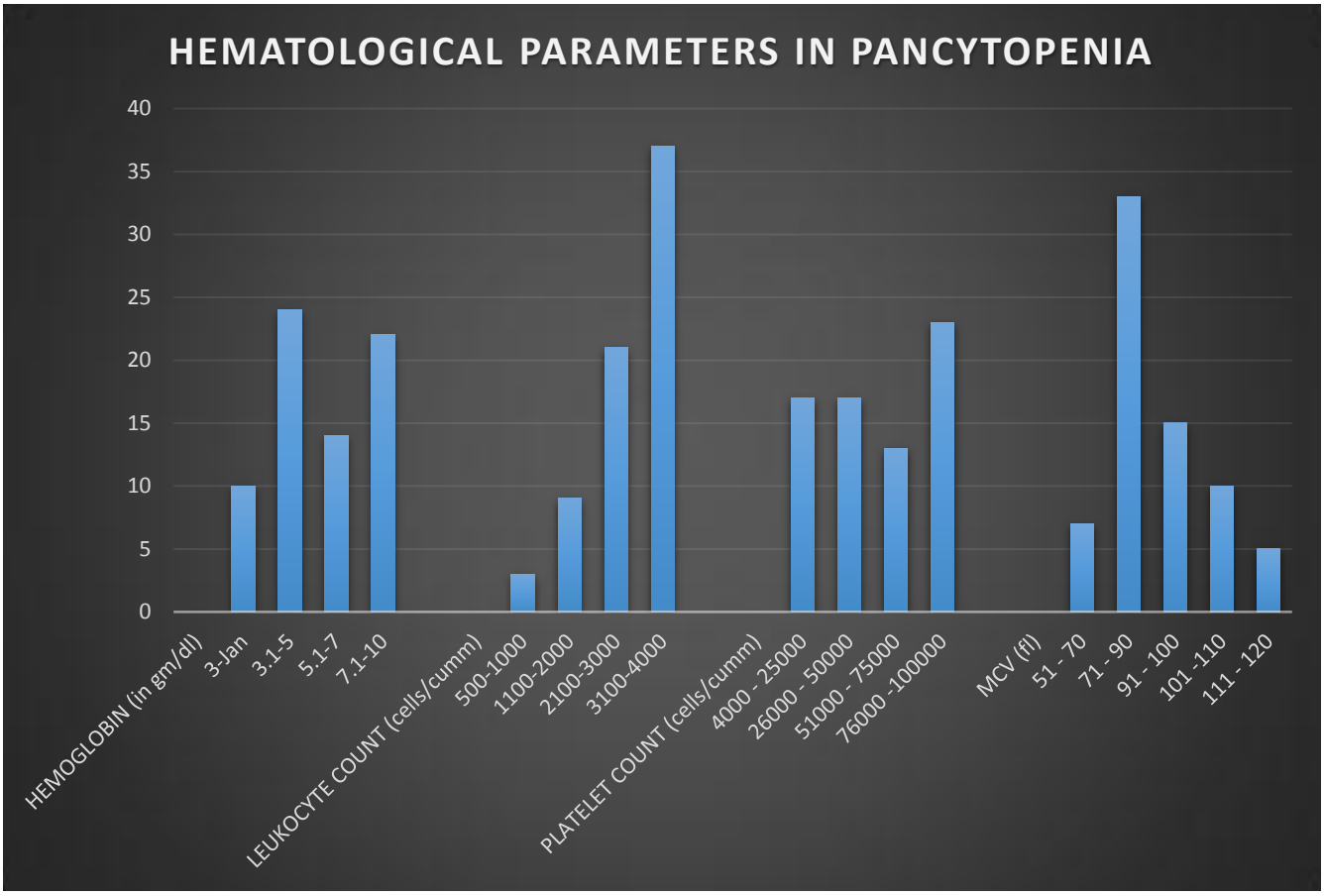


Table: 12 Distribution of peripheral blood smear findings in pancytopenia

PBS	GENDER		TOTAL	PERCENTAGE (in %)
	MALE	FEMALE		
Dimorphic Anemia	13	18	31	44.2
Normocytic Anemia	8	9	17	24.3
Macrocytic Anemia	5	7	12	17.1
Microcytic hypochromic Anemia	3	5	8	11.4
Acute myeloid leukemia – M4	1	0	1	1.4
Hereditary Elliptocytosis	1	0	1	1.4
Total	31	39	70	100

As shown in the table: 12 and figure: 12, 31 (44.2%) of patients had Dimorphic anemic blood picture, and 8 (11.4%) of the cases had microcytic hypochromic anemia followed by one case each of acute leukemia and hereditary elliptocytosis.

Fig: 12 Distribution of peripheral blood smear findings in pancytopenia

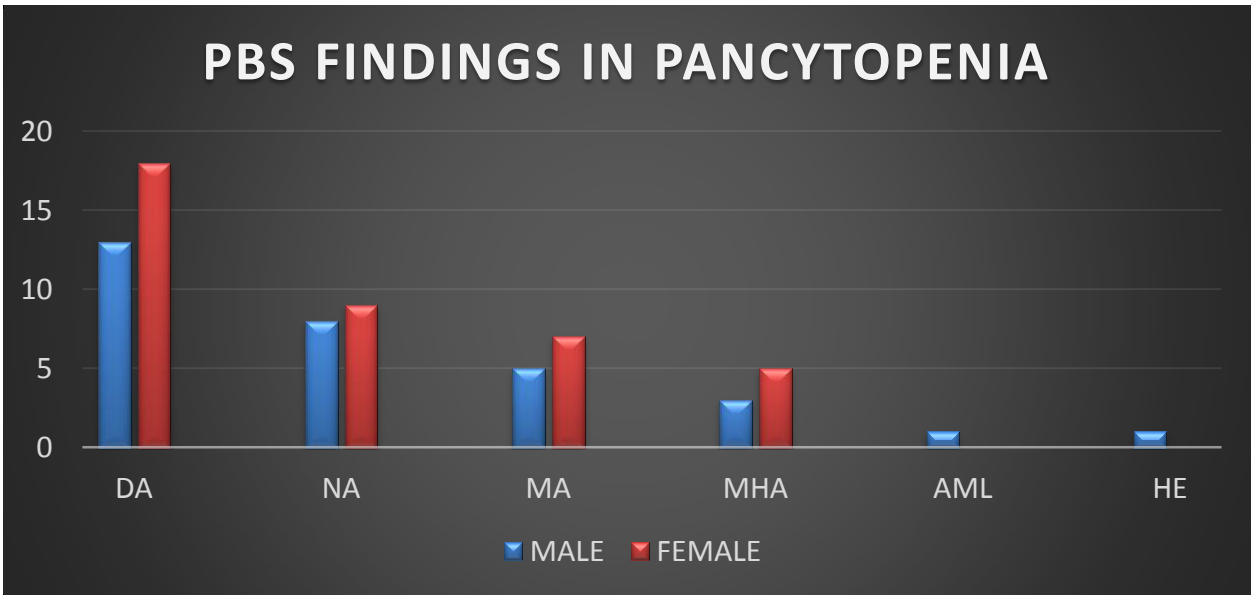


Table: 13 Bone marrow cellularity in patients with pancytopenia

TYPE OF CELLULARITY	NUMBEROF PATIENTS	PERCENTAGE (in %)
Hypocellular	13	18.7
Normocellular	23	32.8
Hypercellular	34	48.5
Total	70	100

Bone marrow aspirate in the present study of pancytopenia showed the following types of cellularity**:** a) Hypocellularity -18.7 %, b) Normocellularity – 32.8 %, c) Hypercellularity- 48.5%

Fig: 13 Bone marrow cellularity in patients with pancytopenia

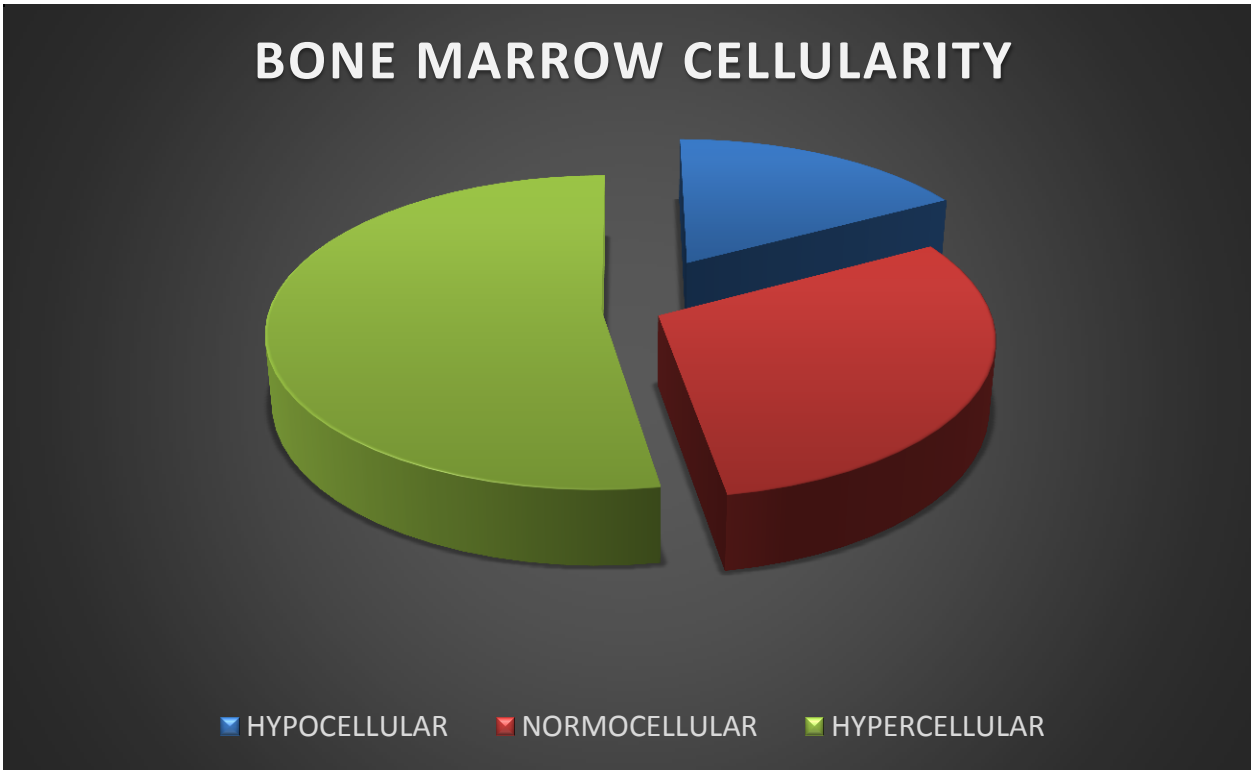


Table: 14 Erythroid series findings in pancytopenia

ERYTHROID SERIES	TOTAL	PERCENTAGE (in %)
Micronormoblastic maturation/megaloblastic maturation	17	24.2
Megaloblastic Maturation	23	32.8
Micronormoblastic maturation	12	17.4
Normoblastic Maturation	18	25.7
TOTAL	70	100

As shown in the table: 14 and figure: 14 megaloblastic maturation was seen in 23 (32.8%) of the patients and 17(24.2%) showed dimorphic maturation pattern (both micronormoblastic and megaloblastic maturation)

Fig: 14 Erythroid series findings in pancytopenia

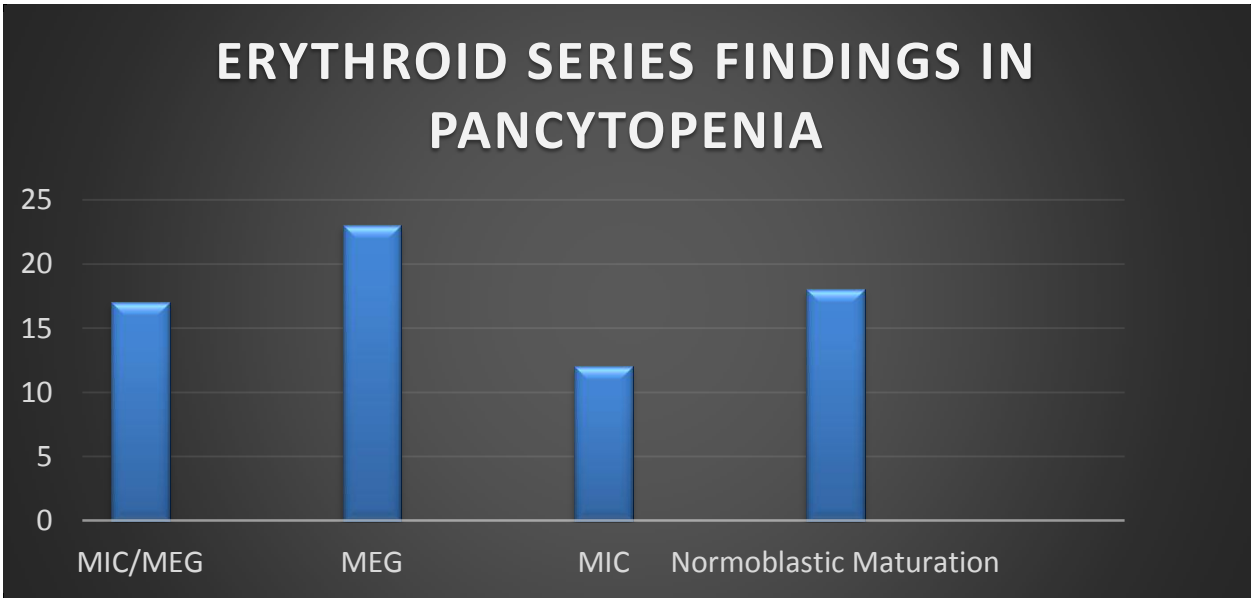


Table: 15 Myeloid series findings in pancytopenia

MYELOID SERIES	TOTAL (n=70)	PERCENTAGE (in %)
Normal	57	81.4
Abnormal	13	18.6
• Giant Metamyelocytes • Blasts • Dysplasia	8 3 2	11.4 4.2 2.8

As shown in the table:15 and figure:15 the myeloid series showed abnormal forms like immature blasts cells were seen in 1 case of acute leukemia and 2 cases myelodysplastic syndrome and dysplastic changes were seen in 2 cases of myelodysplastic syndrome and 8(11.4%) giant Metamyelocytes were seen in megaloblastic anemia.

Fig: 15 Myeloid series findings in pancytopenia

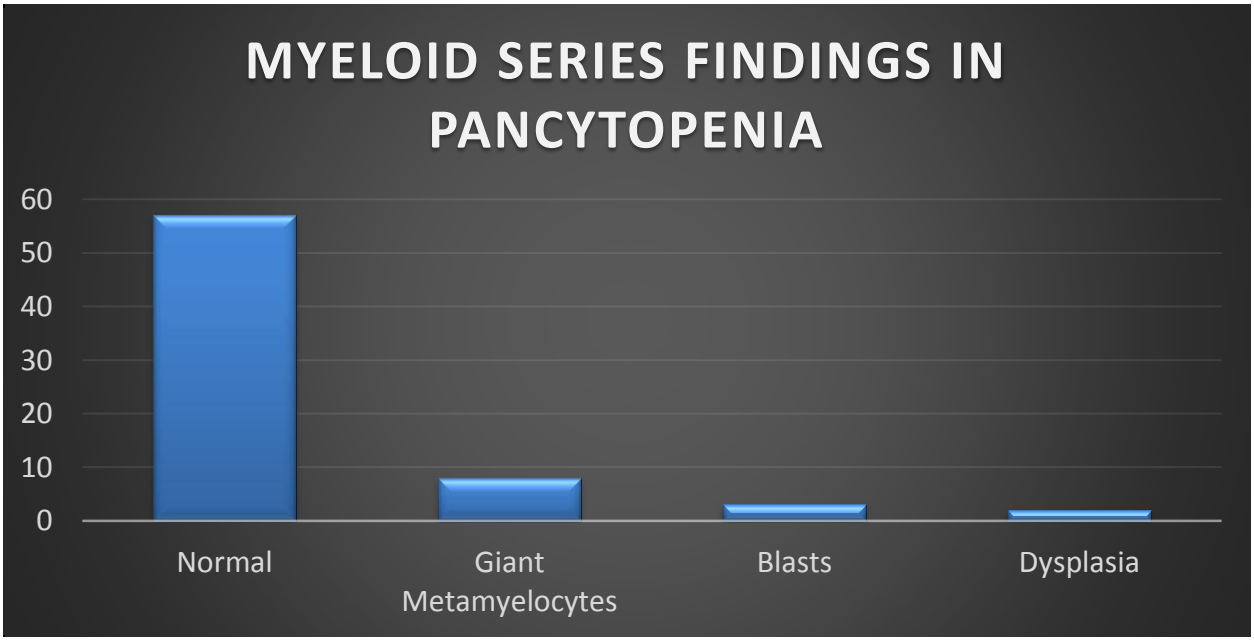
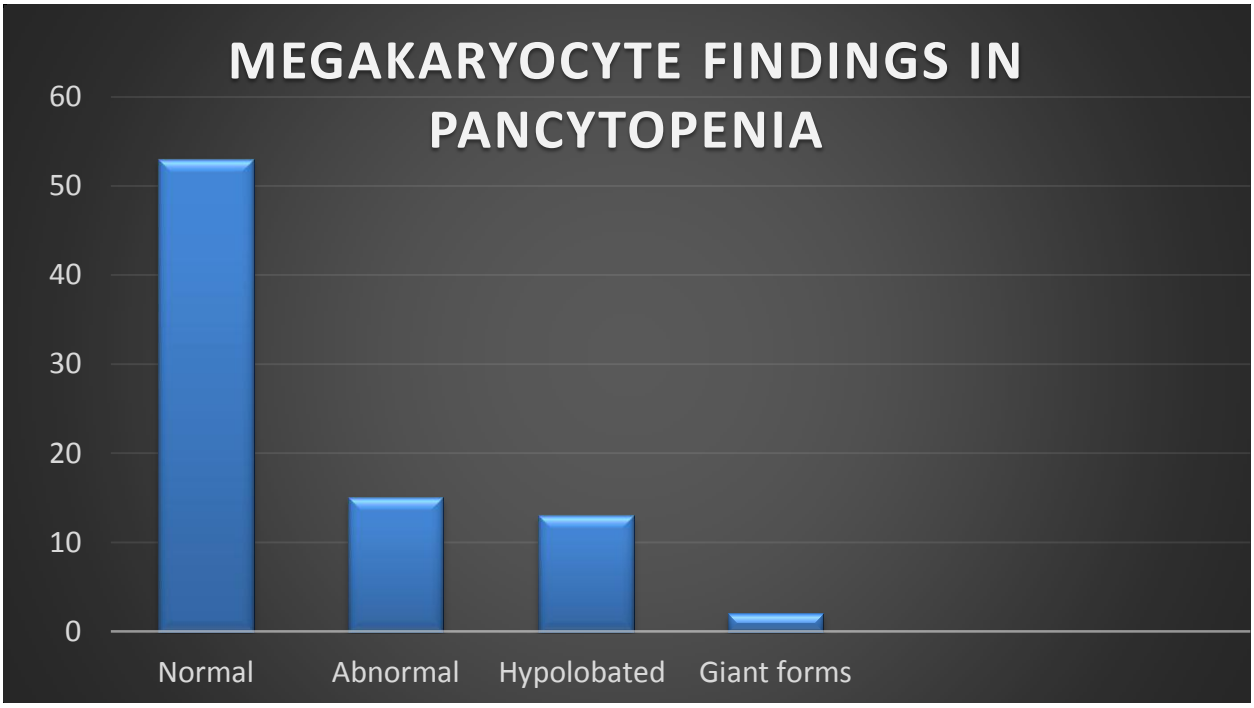


Table: 16 Megakaryocyte findings in pancytopenia

MEGAKARYOCYTE	TOTAL	PERCENTAGE (in %)
Normal	53	75.7
Abnormal	17 (15+2)	24.2
• Hypolobated • Giant forms	15 2	21.4 2.8

As shown in the table: 16 and figure: 16 below megakaryocyte findings in pancytopenia showed normal megakaryocyte morphology in 75.7% of the cases and megakaryocyte abnormality in the form of hypolobation 15(21.4%) and 2(2.8%) giant forms were seen in of all the cases of pancytopenia.

Fig: 16 Megakaryocyte findings in pancytopenia



Further analysis was done under the following headings:

1. Pancytopenia associated with hypocellular bone marrow

2. Pancytopenia associated with hypercellular and normocellular bone marrow

a) Pancytopenia associated with megaloblastic anemia

b) Pancytopenia associated with nutritional anemia

c) Pancytopenia associated with viral infections

d) Pancytopenia associated with malignant diseases- acute leukemia, myelodysplastic syndrome

e) Pancytopenia associated with pure red cell aplasia

f) Pancytopenia associated with hereditary spherocytosis

Table: 17 Pancytopenia based on bone marrow cellularity

BONE MARROW SPECTRUM	HYPO CELLULAR		HYPER CELLULAR		NORMO CELLULAR		TOTAL NUMBER OF CASES (n=70)	
	No	(%)	No.	(%)	No.	(%)	No.	(%)
Megaloblastic anemia	5	38.4	20	64.5	11	42.3	36	44.2
Nutritional anemia	7	53.8	7	22.5	12	46.1	26	27.2
Viral infections			1	3.2	2	7.6	3	4.2
Hereditary elliptocytosis			-	-	1	3.8	1	1.4
Pure red cell aplasia	1	7.6					1	1.4
Acute leukemia			1	3.2	-	-	1	1.4
MDS			2	6.4	-	-	2	3.8
Total	13		31		26		70	100

Hypo cellular bone marrow showed 5 cases of megaloblastic anemia, 7 cases of nutritional anemia and 1 cases of pure red cell aplasia. Hypercellular marrow showed 20 cases of megaloblastic anemia and 7 cases of nutritional anemia 2 cases of myelodysplastic syndrome and one case each of acute leukemia and viral infection, while in was normocellular marrow showed 11 cases of megaloblastic anemia ,12 cases of nutritional anemia, 2 cases of viral infections and 1 case of hereditary elliptocytosis as shown in the above table:17 and figure:17 Megaloblastic anemia (44.2%) was the most common bone marrow finding followed by nutritional anemia (27.2%), viral infections (4.2%) .Table17 and figure: 17 a, 17 b, 17 C.

Fig: 17a Bone marrow spectrum in pancytopenia

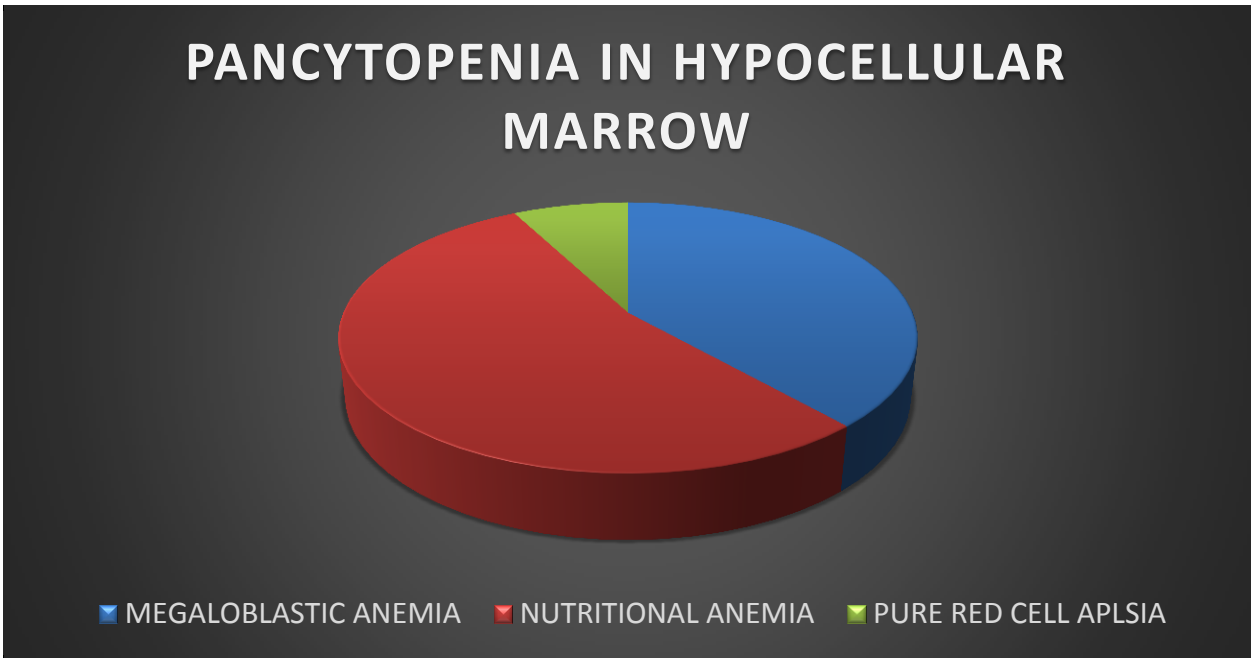


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Fig: 17b Bone marrow spectrum in pancytopenia

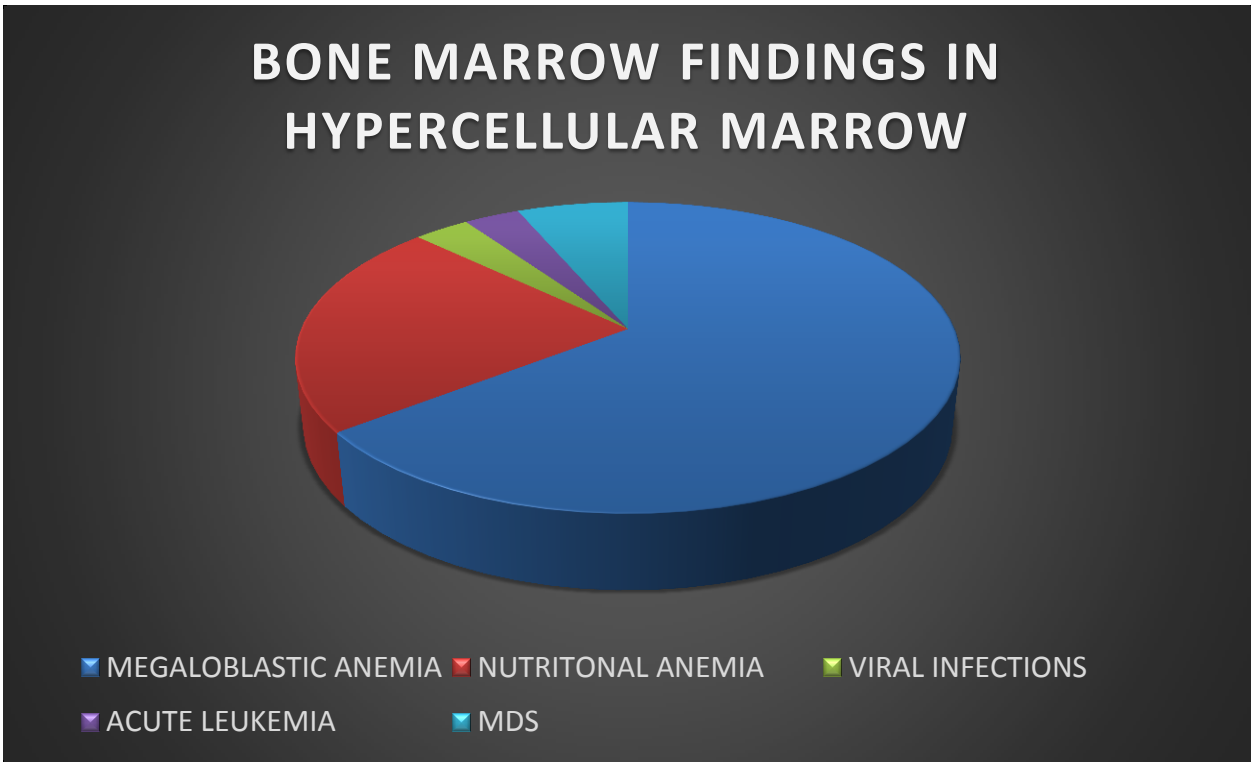
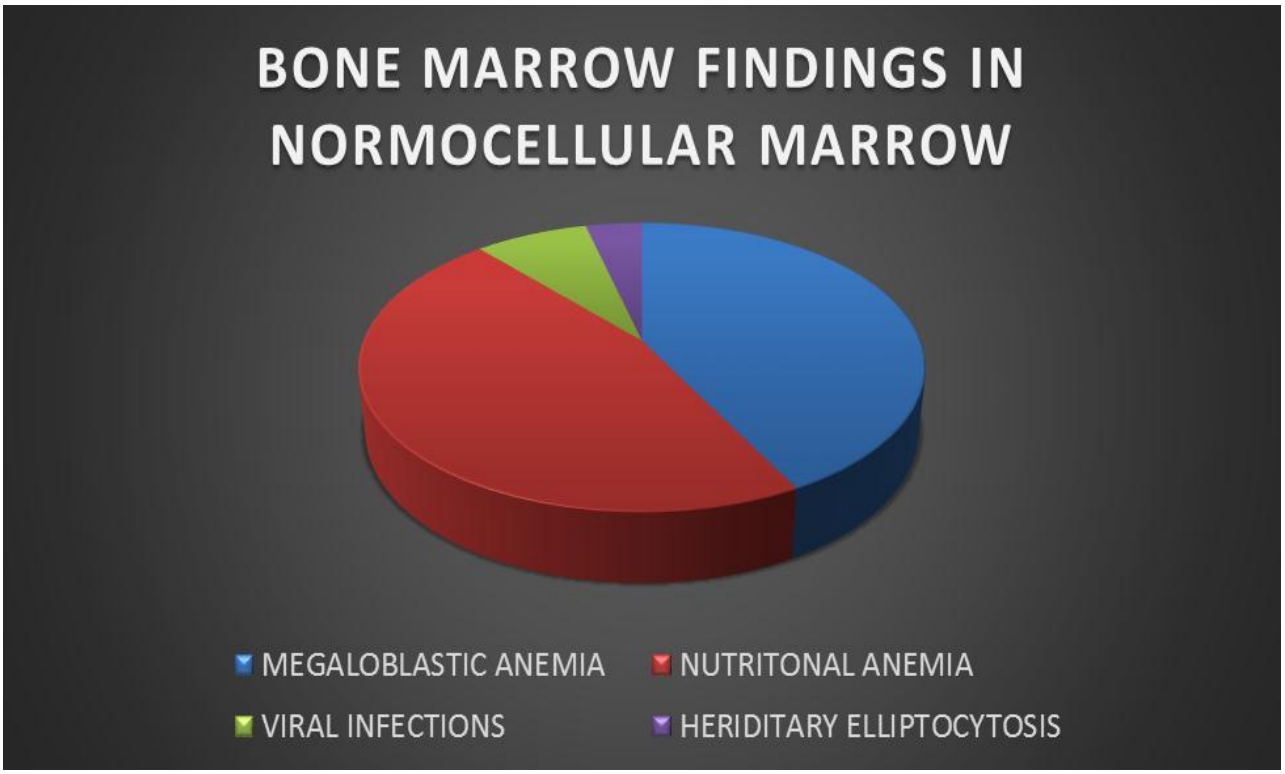


Fig: 17c Bone marrow spectrum in pancytopenia



PANCYTOPENIA WITH MEGALOBLASTIC ANEMIA

In the present study 36 cases of megaloblastic anemia were seen. It constituted 64.5% of cases with hypercellular marrow and 42.3% of cases with normocellular marrow and 38.4% of cases with hypocellular marrow.

Table: 18 Age and sex distribution of patients with megaloblastic anemia

AGE (in years)	GENDER		TOTAL	PERCENTAGE (in %)
	MALE	FEMALE		
<20	3	4	7	10
20-30	4	4	8	11.4
31-40	1	3	4	5.7
41-50	2	2	4	5.7
51-60	7	3	10	14.2
>60	0	3	3	4.2
TOTAL	17	29	36	100

As shown in the table: 18 and figure: 18 below, megaloblastic anemia was seen to occur in 10 (14.2%) of the cases in the age group 51-60 years. There was female preponderance and female to male ratio was 1.7:1.

Fig: 18 Age and sex distribution of patients with megaloblastic anemia

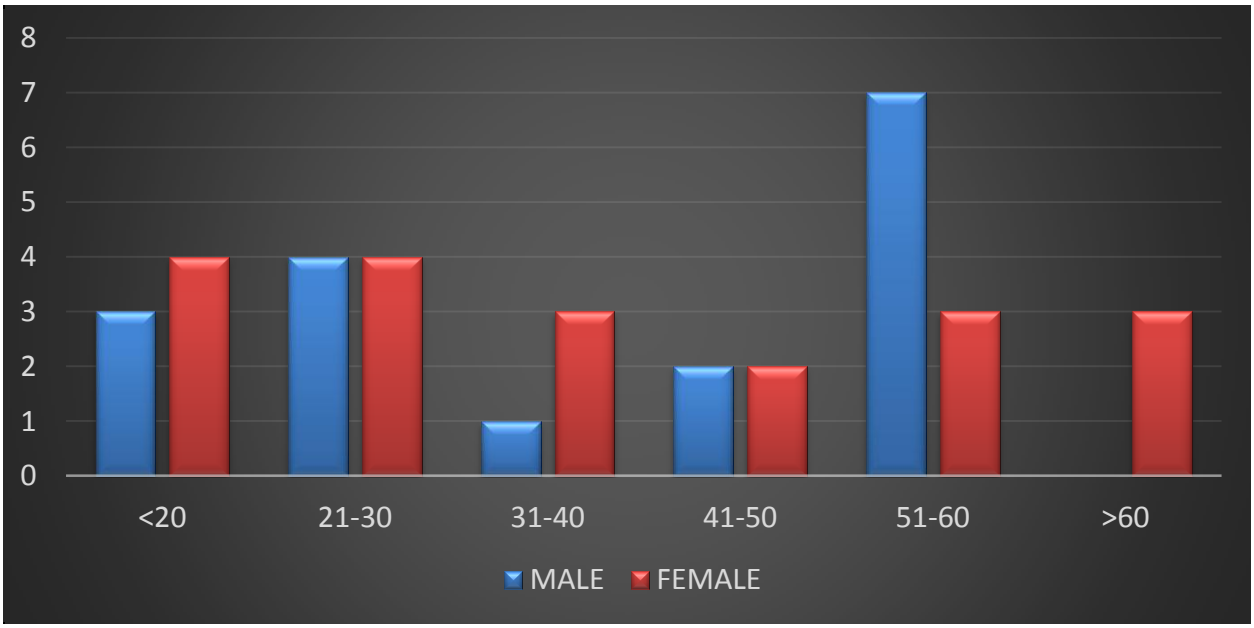


Table: 19 Spectrum of symptoms in megaloblastic anemia

SYMPTOMS	NUMBER OF CASES	PERCENTAGE (in %)
Generalised weakness	21	30
Fever	25	35.7
Easy fatigubility	27	38.5
Pain abdomen	4	5.7
Bleeding tendencies	3	4.2
Breathlessness	2	2.8

As shown in the table: 19 and figure: 19, the patients with bone marrow spectrum showing megaloblastic anemia presented with easy fatigubility in majority 27(38.5%) of the cases.

Fig: 19 Spectrum of symptoms in megaloblastic anemia

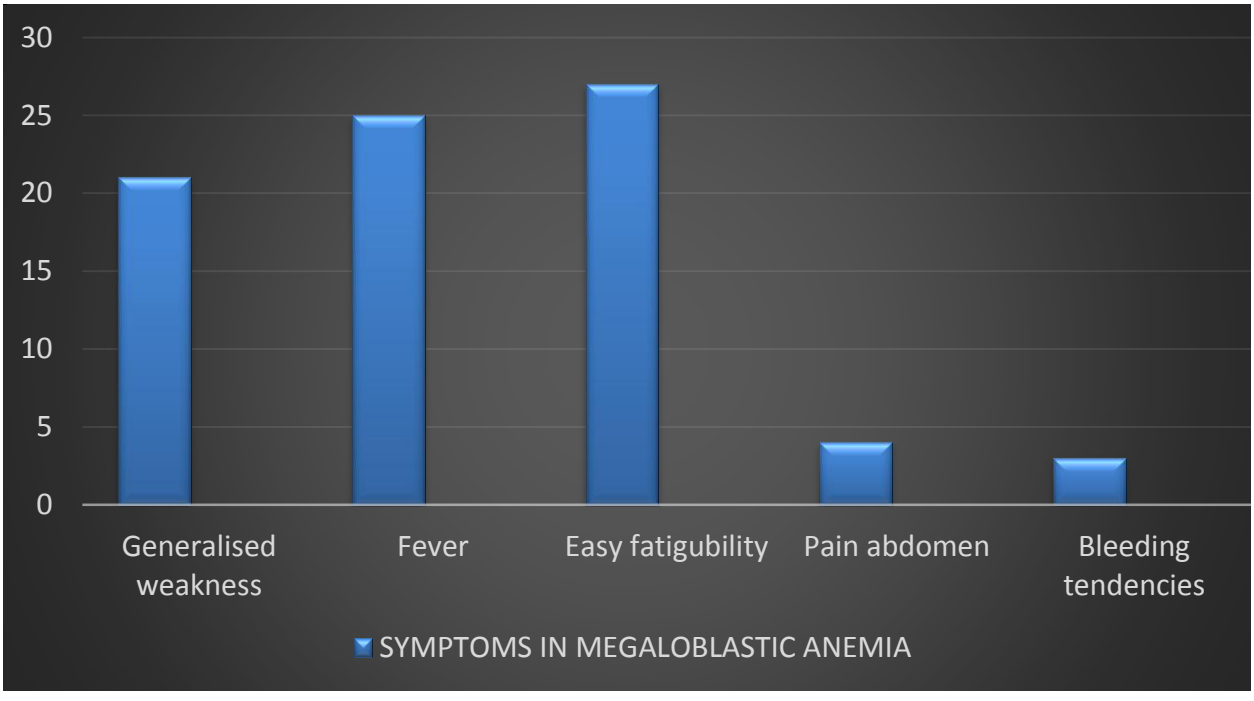


Table: 20 Spectrum of signs in megaloblastic anemia

SIGNS	NUMBER OF CASES	PERCENTAGE (in %)
Pallor	32	45.7
Icterus	4	5.7
Edema	4	5.7
Lymphadenopathy	1	1.4
Splenomegaly	5	7.1
Hepatomegaly	4	5.7

As shown in the table: 20 and figure: 20, the patients with bone marrow spectrum showing megaloblastic anemia had majority 32(45.7%) of the cases with pallor as the most common sign.

Fig: 20 Spectrum of signs in megaloblastic anemia

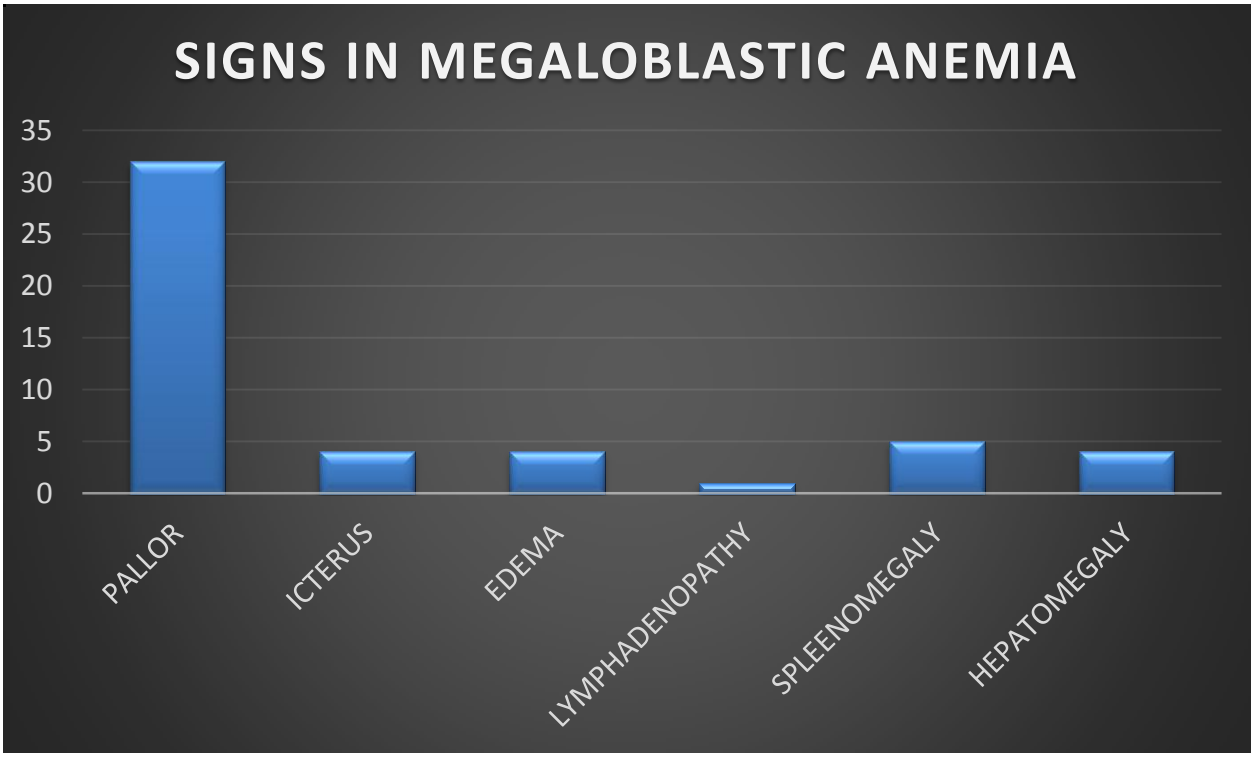


Table: 21 Distribution of peripheral blood smear in megaloblastic anemia

PBS	NUMBER OF CASES	PERCENTAGE (in %)
Dimorphic Anemia	25	50
Macrocytic Anemia	8	11.4
Normocytic Anemia	3	4.2
Total	36	100

As shown in the table:21 and figure:21, the patients with bone marrow spectrum showing megaloblastic anemia showed Dimorphic anemia on peripheral blood smear in majority 25(50%) of the cases. The least common finding was normocytic anemia seen in 3(4.2%) of the cases.

Fig: 21 Distribution of peripheral blood smear in megaloblastic anemia

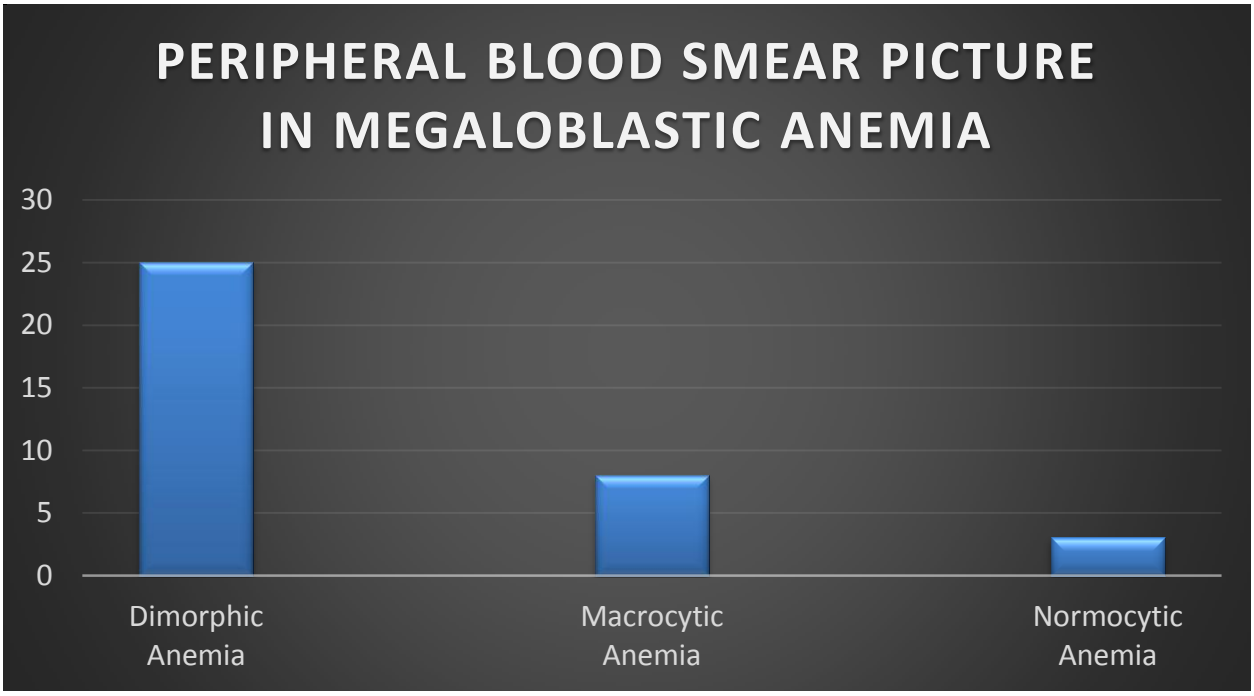
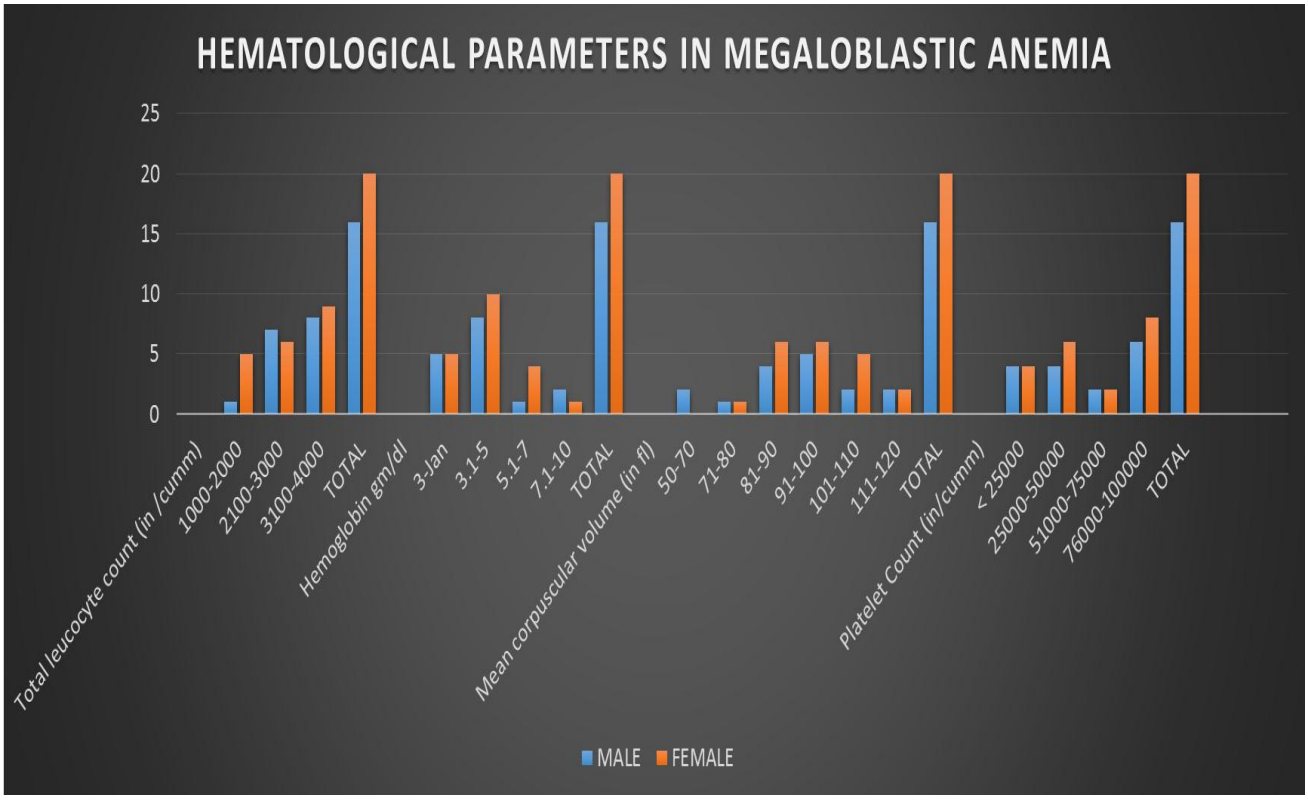


Table: 22 Hematological parameters studied in megaloblastic anemia

HEMATOLOGICAL PARAMETERS	GENDER		TOTAL	PERCENTAGE (In %)
	MALE	FEMALE		
Total leucocyte count (in /cumm)				
1000-2000	1	5	6	8.5
2100-3000	7	6	13	18.5
3100-4000	8	9	17	24.2
TOTAL	16	20	36	100
Hemoglobin in gm %				
1-3	5	5	10	14.2
3.1-5	8	10	18	25.7
5.1-7	1	4	5	7.1
7.1-10	2	1	3	4.2
TOTAL	16	20	36	100
Mean corpuscular volume (in fl)				
50-70	2	0	2	2.8
71-80	1	1	2	2.8
81-90	4	6	10	14.2
91-100	5	6	11	15.7
101-110	2	5	7	10
111-120	2	2	4	5.7
TOTAL	16	20	36	100
Platelet Count (in/cumm)				
< 25000	4	4	8	11.4
25000-50000	4	6	10	14.2
51000-75000	2	2	4	5.7
76000-100000	6	8	14	20
TOTAL	16	20	36	100

As shown in the table:22 and figure:22, 17(24.2%) of the cases showed maximum leucocyte range of 3100-4000/cumm and minimum leucocyte range was 1000-2000/cumm seen in 6(8.5%) of the cases. 18(25.7%) showed maximum hemoglobin range of 3.1-5 gm/dl and minimum hemoglobin range was 1-3 gm/dl seen in 10(14.2%) of the cases. 14(20%) cases showed maximum platelet count range of 76,000-1,00,00 and minimum platelet count range was 51,000-75,000/cumm seen in 4(5.7%) of the cases.11(15.7%) of cases showed a maximum MCV range of 91-100 fl.

Fig: 22 Hematological parameters studied in megaloblastic anemia



Findings of erythroid series in megaloblastic anemia

Erythroid Series in megaloblastic anemia showed mainly megaloblastic maturation with megaloblasts showing pale blue cytoplasm and large nucleus with sieve like chromatin and exhibiting nucleo-cytoplasmic asynchrony.

Table: 23 Dyserythropoiesis in megaloblastic anemia

DYSERYTHROPOIESIS	GENDER		TOTAL	PERCENTAGE (in %)
	MALE	FEMALE		
Budding forms and binucleate forms	8	7	15	21.4

As shown in the table:23 and figure:23, the patients with bone marrow spectrum showing megaloblastic anemia showed dyserythropoeisis in 15 cases in the form of budding forms and binucleate forms. Out of which 8 males and 7 females showed features of dyerythropoeisis.However, this excluded dyserythropoiesis seen in myelodysplastic syndrome

Fig: 23 Dyserythropoiesis in megaloblastic anemia

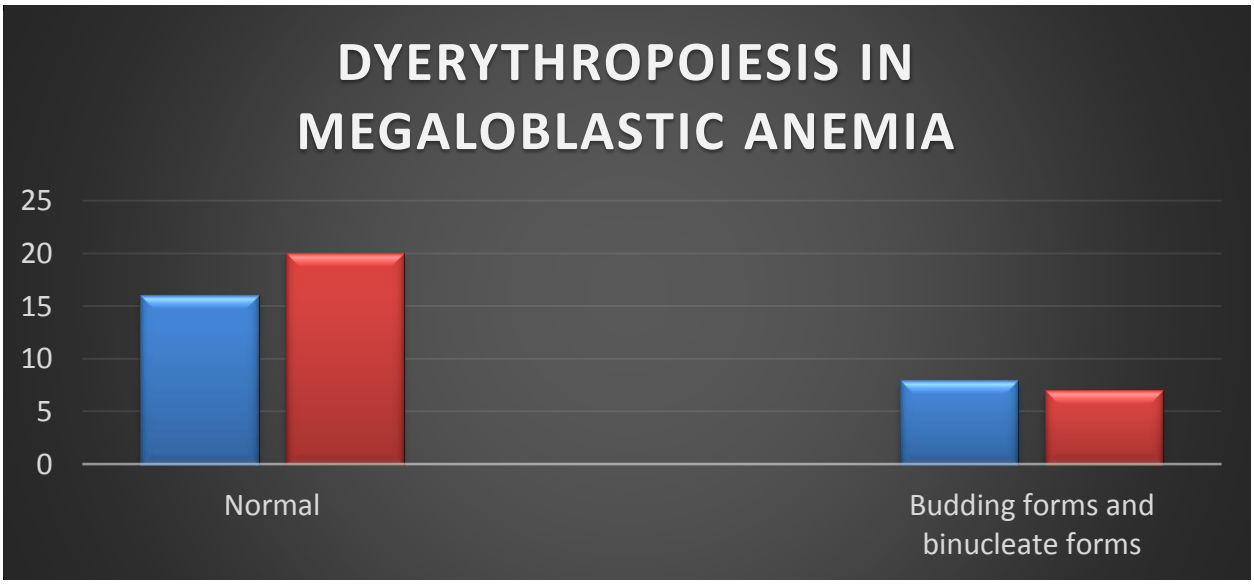


Table: 24 Findings of myeloid series in megaloblastic anemia

MYELOID	NUMBER OF CASES	PERCENTAGE (in %)
Normal morphology	28	40
Giant Metamyelocytes	8	11.4

As shown in the table: 24 and figure: 24, the patients with bone marrow spectrum showing megaloblastic anemia the myeloid series showed most commonly normal morphology. 18(11.4%) of the cases showed giant metamyelocytes.

Table: 24 Findings of myeloid series in megaloblastic anemia

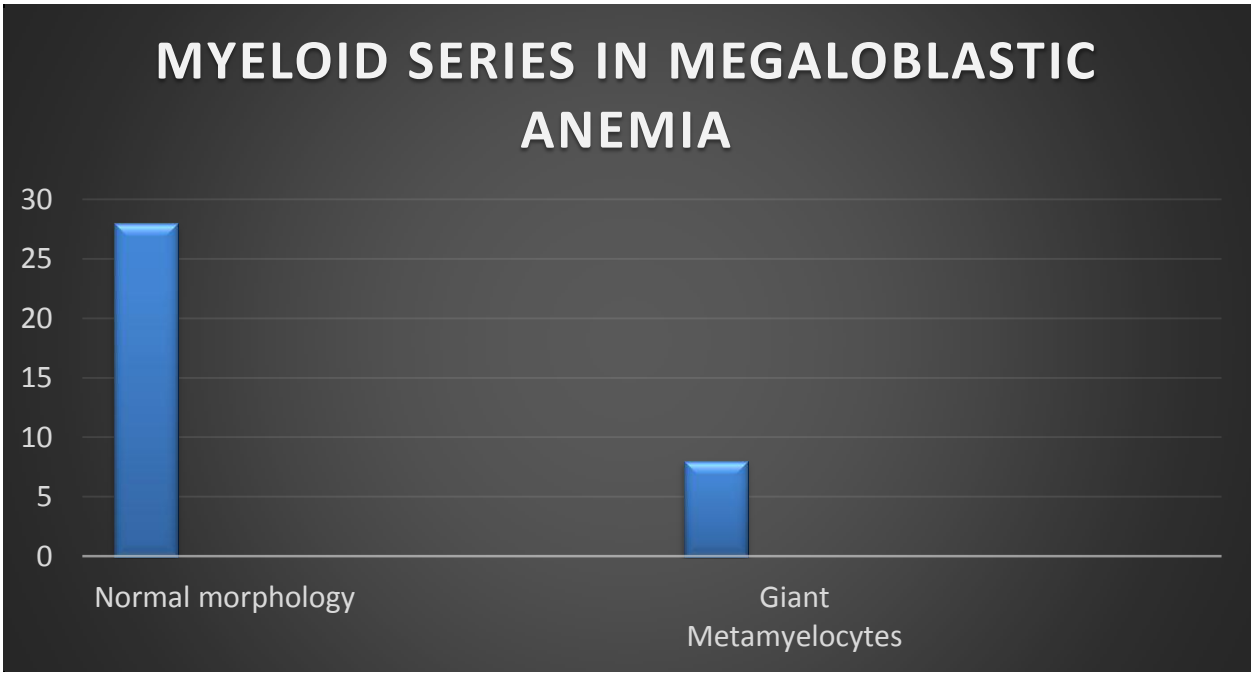


Table: 25 Findings of megakaryocyte series in megaloblastic anemia

MEGAKARYOCYTE	TOTAL (n=70)	PERCENTAGE (in %)
Normal	53	75.7%
Abnormal	11	15.7
• Hypolobated • Giant forms	10 1	14.2 1.4

As shown in the above table: 25 and figure: 25, the patients with bone marrow spectrum showing megaloblastic anemia showed abnormal megakaryocytes in 11(15.7%) of the cases. Out of which 10 (14.2%) cases showed hypolobated forms.

Fig: 25 Findings of megakaryocyte series in megaloblastic anemia

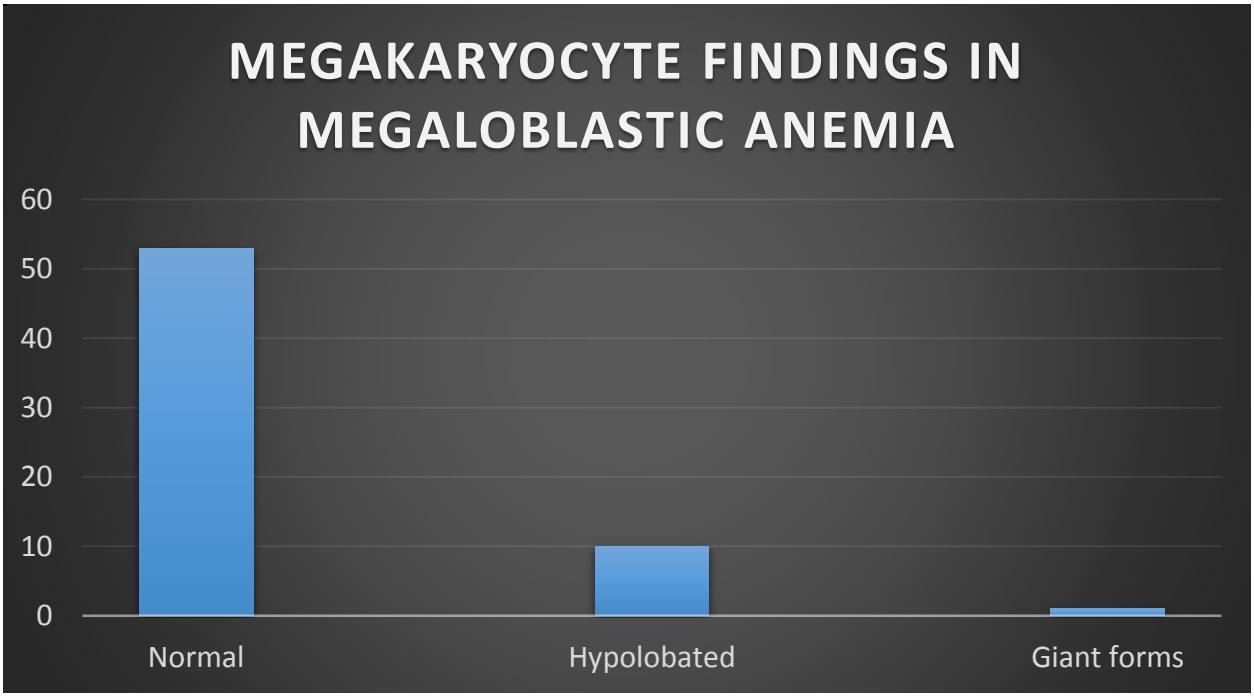


Table: 26 Age and Sex distribution in mixed nutritional anemia

AGE (in years)	GENDER		TOTAL	PERCENTAGE (in %)
	MALE	FEMALE		
<20	1	2	3	4.2
20-30	1	4	5	7.1
31-40	3	3	6	8.5
41-50	2	3	5	7.1
51-60	1	1	2	2.8
>60	4	3	7	10
TOTAL	12	16	28	100

As shown in the table: 26 and figure: 26, the patients with bone marrow spectrum showing nutritional anemia majority 7(10%) of the cases were more than 60 years of age. The female to male ratio was 1.3:1.

Fig: 26 Age and Sex distribution in mixed nutritional anemia

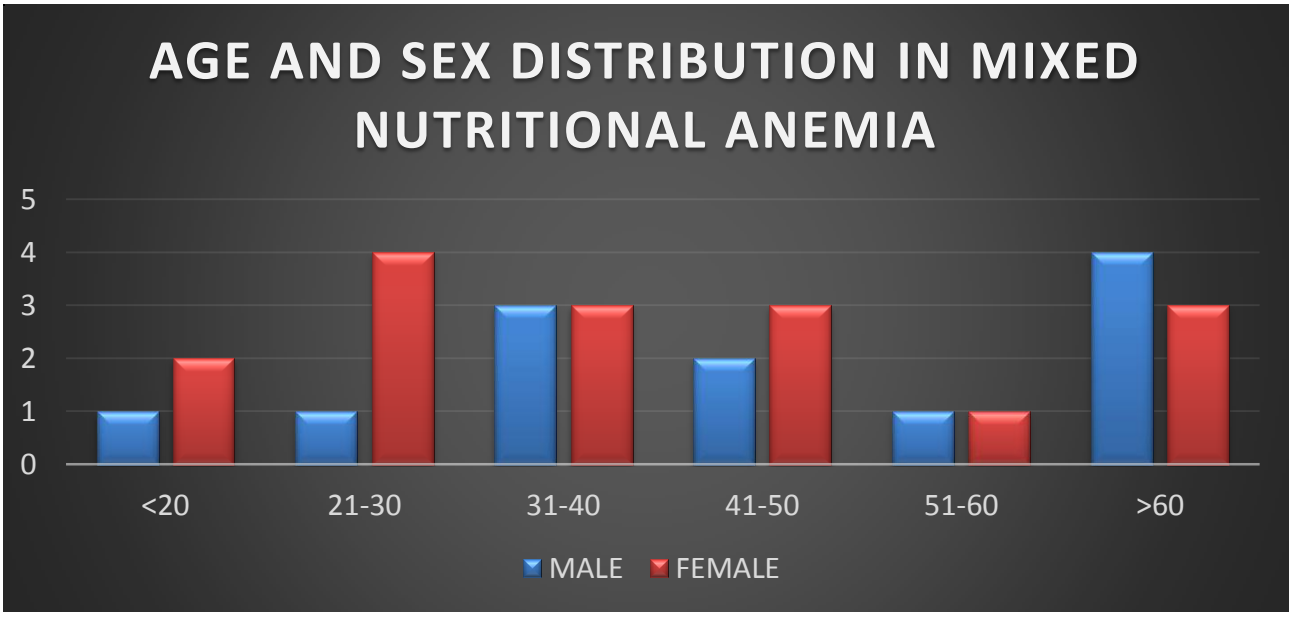


Table: 27 Spectrum of symptoms in mixed nutritional anemia

SYMPTOMS	NUMBER OF CASES	PERCENTAGE (in %)
Generalised weakness	14	20
Fever	20	28.7
Easy fatigubility	6	8.5
Pain abdomen	3	4.2
Bleeding tendencies	7	10
Altered sensorium	1	1.4

As shown in the table: 27 and figure: 27, the patients with bone marrow spectrum showing Nutritional Anemia had majority 20(28.7%) of the cases presented with fever, followed by generalized weakness seen in 14(20%) and bleeding tendencies seen in 7(10%) of the patients.

Fig: 27 Spectrum of symptoms in mixed nutritional anemia

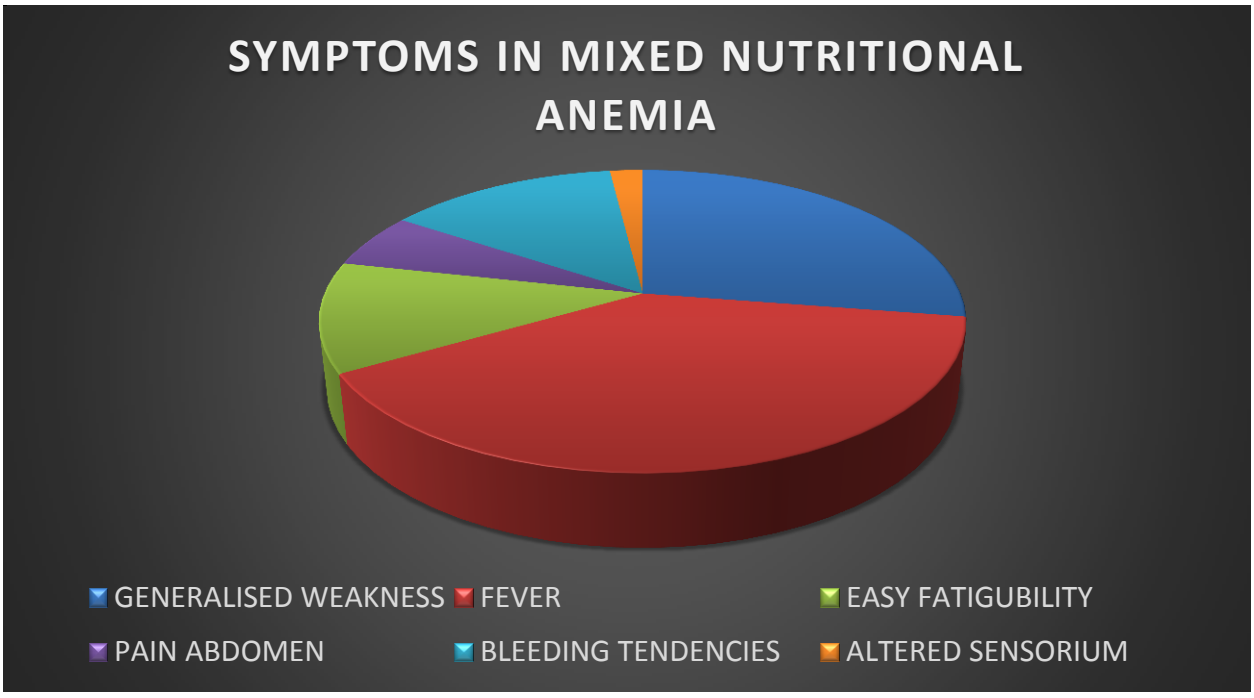


Table: 28 Spectrum of signs in mixed nutritional anemia

SIGNS	TOTAL	PERCENTAGE (in %)
Pallor	30	42.8
Icterus	2	2.85
Edema	2	2.85
Splenomegaly	4	5.7
Hepatomegaly	2	2.85

As shown in the table: 28 and figure: 28, the patients with bone marrow spectrum showing nutritional anemia had pallor as the commonest presenting sign in majority 30 (42.8%) of the cases.

Table: 28 Spectrum of signs in mixed nutritional anemia

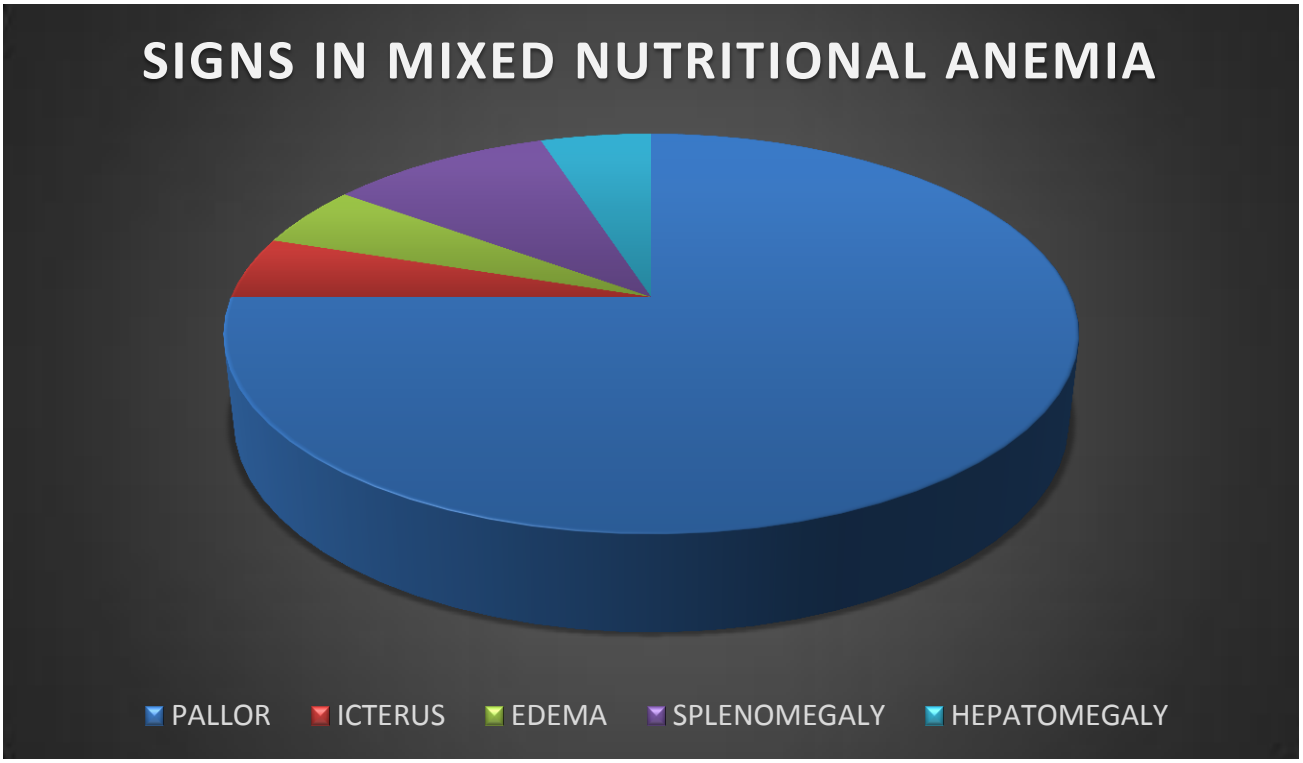


Table: 29 Range of hematological parameters in mixed nutritional anemia

HEMATOLOGICAL PARAMETERS	GENDER		TOTAL	PERCENTAGE (In %)
	MALE	FEMALE		
Total leucocyte count (in /cumm)				
• 1000-2000	2	1	3	4.2
• 2100-3000	1	7	8	11.4
• 3100-4000	3	12	15	21.4
Hemoglobin (in gm/dl)				
• 1-3	5	6	11	15.7
• 3.1-5	1	6	7	10
• 5.1-7	0	2	2	2.8
• 7.1-10	3	3	6	8.5
Mean corpuscular volume(in fl)				
• 50-70	2	3	5	7.1
• 71-80	2	5	7	10
• 81-90	5	4	9	12.8
• 91-100	0	1	1	1.4
• 101-110	2	1	3	4.2
• 111-120	1	0	1	1.4
Platelet Count (in /cumm)				
• < 25000	1	5	6	8.5
• 25000-50000	3	2	2	2.8
• 51000-75000	1	6	5	7.4
• 76000-100000	4	4	7	10

As shown in the table:29 and figure:29, 15(21.4%) of the cases showed maximum leucocyte range of 3100-4000/cumm and minimum leucocyte range was 1000-2000/cumm seen in 3(4.2%) of the cases. 11(15.7%) showed maximum hemoglobin range of 1-3 gm/dl. 7(10%) cases showed

maximum platelet count range of 76,000-1,00,00/cumm. 11(15.7%) of cases showed significant MCV range of 81-90 fl.

Table: 29 Range of hematological parameters in mixed nutritional anemia

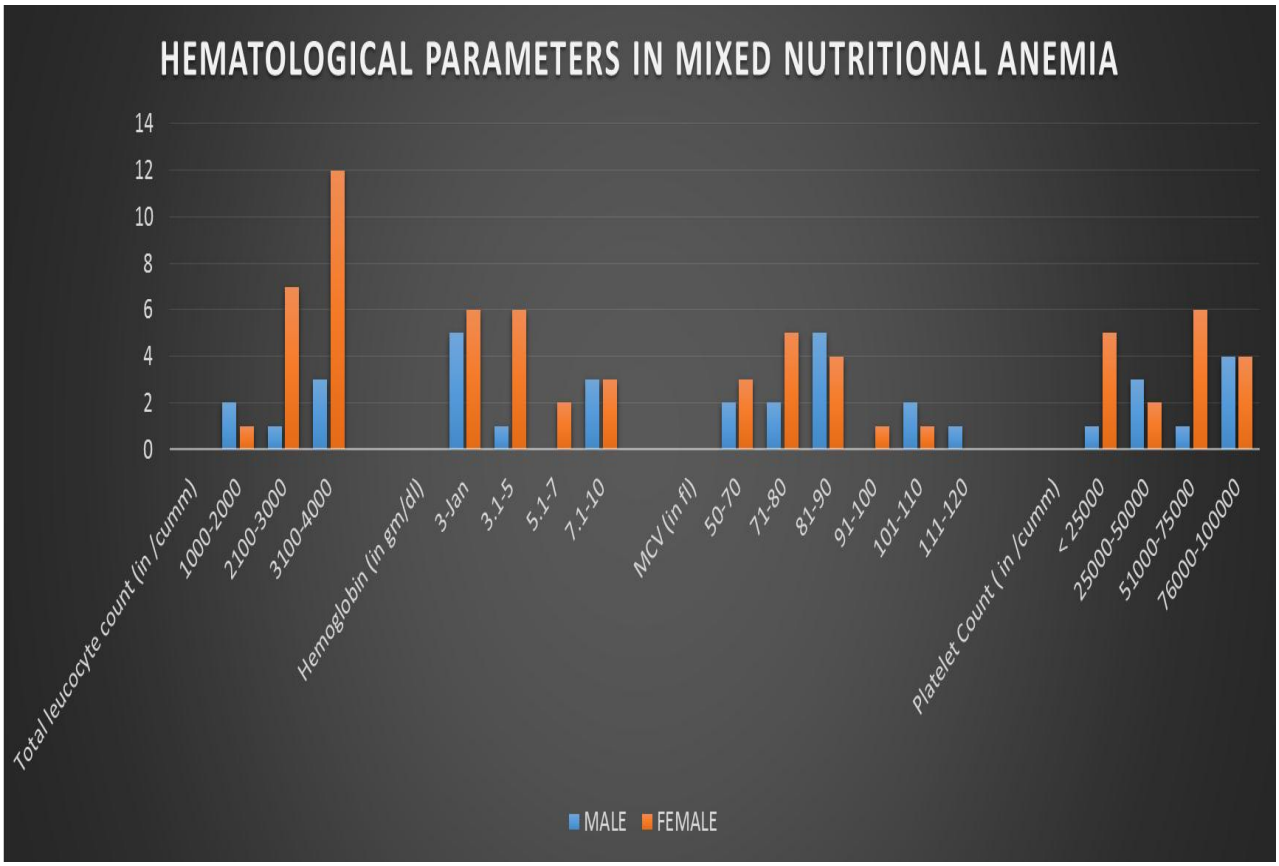


Table: 30 Peripheral blood smear (PBS) findings in mixed nutritional anemia

PBS	TOTAL	PERCENTAGE (in %)
Dimorphic Anemia	10	14.2
Microcytic hypochromic Anemia	7	10
Normocytic Anemia	7	10
Macrocytic Anemia	2	2.8

As shown in the table:30 and figure:30, the patients with bone marrow spectrum showing nutritional anemia the common peripheral blood smear finding was dimorphic anemia seen in 10(14.2%) of the cases followed by microcytic hypochromic anemia and normocytic anemia seen in 7(10%) of the cases each.

Fig: 30 Peripheral blood smear (PBS) findings in mixed nutritional anemia

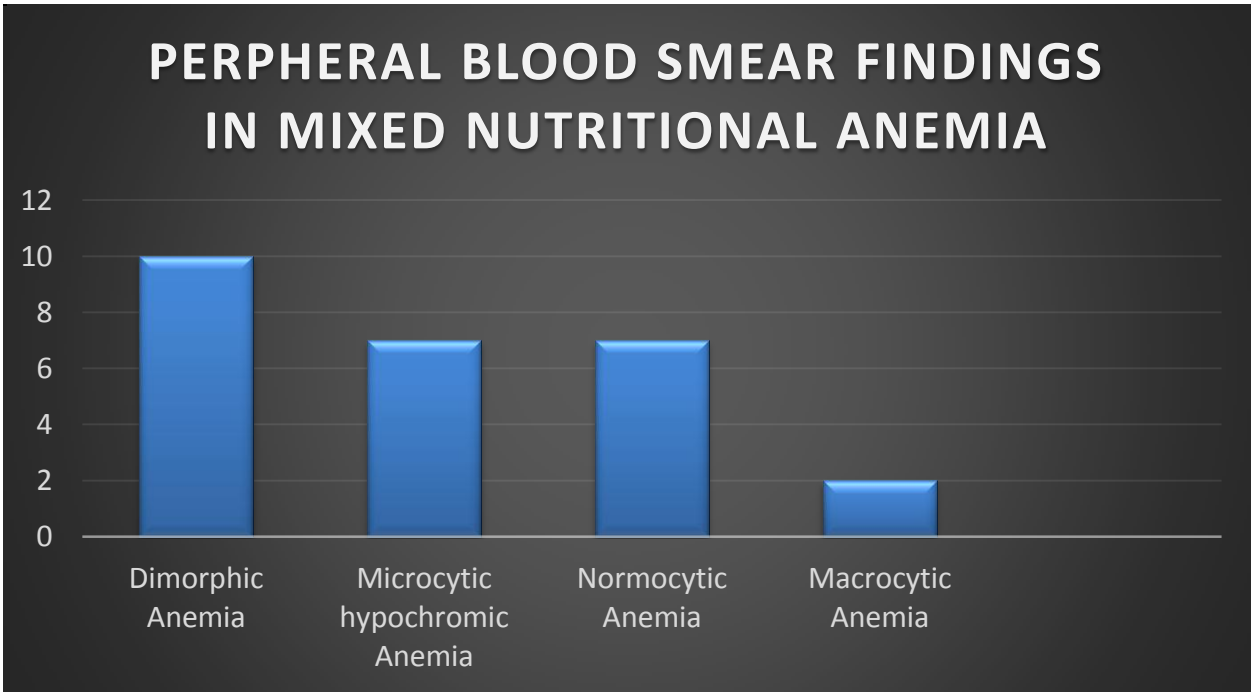
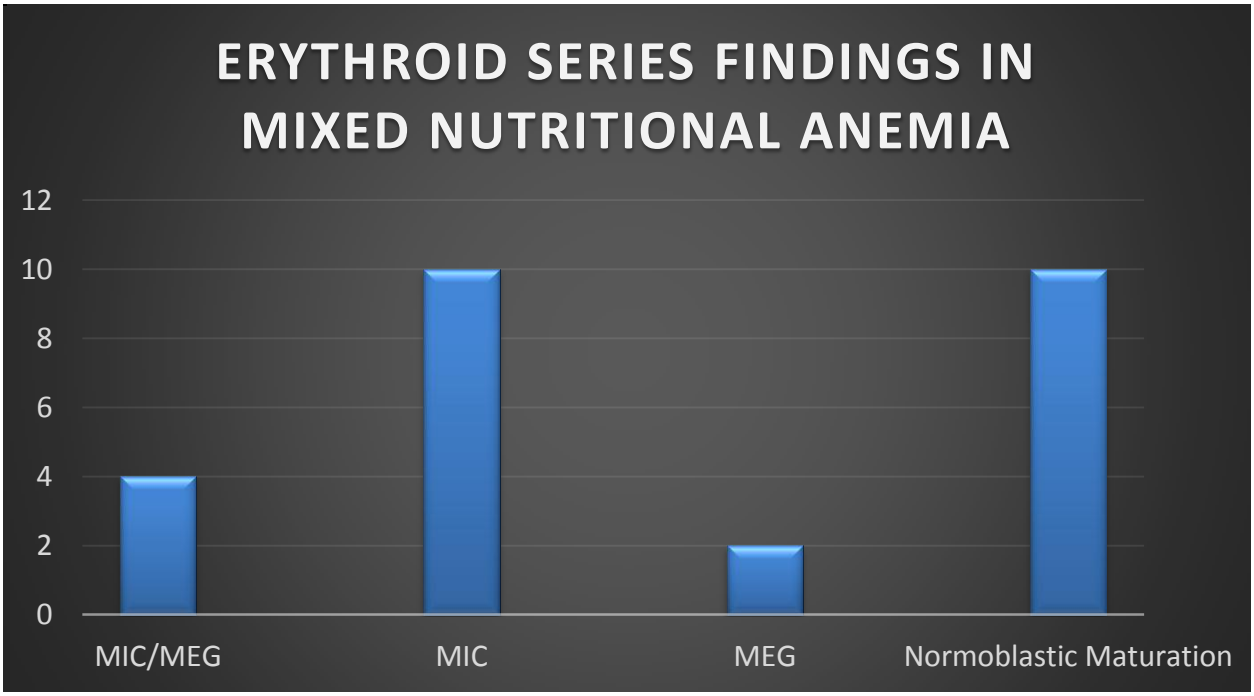


Table: 31 Bone marrow findings in mixed nutritional anemia

ERYTHROID SERIES	TOTAL	PERCENTAGE (in %)
MicronormoblasticMaturation/ Megaloblastic Maturation	4	5.7
Micronormoblastic Maturation	10	14.2
Megaloblastic Maturation	2	2.8
Normoblastic Maturation	10	14.2
TOTAL	26	100
MYELOID SERIES	**TOTAL**	
Normal morphology	26	
MEGAKARYOCYTES	**TOTAL**	
Normal morphology	26	

Table: 31 Bone marrow findings in mixed nutritional anemia



As shown in the table:31 and figure:31, the patients with bone marrow spectrum showing Nutritional Anemia Erythroid series showed both Micronormoblastic and megaloblastic maturation, micronormoblastic maturation, megaloblastic maturation and normoblastic maturation Majority10 (14.2%) of the patients had micronormoblastic maturation. Myeloid series showed normal morphology. Megakaryocyte showed normal morphology.However, two cases showed decrease in megakaryocyte count and two other cases showed increase in the megakaryocyte count in the bone marrow examination findings.

The Vitamin B12, folate and iron studies were not done in this study.

Table: 32 PANCYTOPENIA WITH VIRAL INFECTIONS

	CASE 1	CASE 2	CASE 3
AGE	40 years	60 years	60 years
SEX	Male	Male	Male
SYMPTOMS	Fever with chills and generalised weakness	Fever and petechial hemorrhages	Fever, easy fatigubility, gum bleeding and pain abdomen
SIGNS	Pallor	Pallor, edema	Pallor
TLC(in/cumm)	500	3,800	3,800
HB (gm/dl)	6.4	9.9	8
MCV (in fl)	76	72	75
PLATELET COUNT	29,000/cumm	10,000/cumm	20,000/cumm
PBS FINDINGS	Normocytic anemia with atypical lymphocytes	Normocytic anemia with atypical lymphocytes	Normocytic anemia with atypical lymphocytes
BONE MARROW FINDINGS	Normocellular marrow with normoblastic maturation	Normocellular marrow with normoblastic maturation	Normocellular marrow with normoblastic maturation

n the present study, there were three cases were categorized as viral infections; Table: 32

These cases showed obvious atypical lymphocytes on peripheral blood smear with white blood cells decreased in count and normal in morphology and platelets were reduced in number.Bone marrow aspiration in all of these cases revealed normocellular marrow with erythroid hyperplasia and normoblastic maturation. Myeloid series was normal in morphology. Megakaryocytes were increased in number and normal in morphology.

PANCYTOPENIA WITH MYELODYSPLASTIC SYNDROME

In the present study, two cases of myelodysplastic syndrome was observed. One case was of 40 year old male with fever and generalized weakness. Total count was 1200/cumm, hemoglobin was 7.3 gm/dl, mean corpuscular volume was 79 fl, and platelet count was 78,000/cumm. Peripheral blood smear erythrocytes showed normocytes with hypochromasia, white blood cells was decreased in count, platelets was also reduced in number. Bone marrow aspiration was done and revealed hypercellular marrow with dyserythropoeisis and M:E ratio-10:1.Erythroid series showed megaloblastic maturation,also seen were certain dyserythropoietic features like nuclear fragmentation, budding multiple nuclei and hyperlobulation. Myeloid series were reduced in number with few atypical cells seen constituting around 9% of blasts.Megakaryocytes showed giant forms and hypolobulated forms. Hence, a diagnosis of myelodysplastic syndrome RAEB-1 was made.

Another case was of 60 year old male with generalized weakness and splenomegaly.Total count was 1000/cumm, hemoglobin was 6 gm/dl, mean corpuscular volume was 86 fl, platelet count was 48,000/cumm. Peripheral blood smear erythrocytes showed normocytes with hypochromasia, white blood cells were decreased in count and platelets were reduced in number.

Bone marrow aspiration was unsuccessful hence bone marrow biopsy was performed under aseptic precautions which revealed hyper cellular marrow, M: E ratio could not be assessed. Erythroid series showed megaloblastic maturation with dyserythropoeisis.Myeloid series showed immature granulocytes. Megakaryocytes were increased in number and showed dysplastic changes, hypolobated forms were also seen. Additionally eosinophils and plasma cells were also seen. Hence a diagnosis of Myelodysplastic marrow was confirmed

PANCYTOPENIA WITH ACUTE LEUKEMIA

In the present study, one case of acute leukemia was observed presenting with pancytopenia. A 24 year old female with generalized weakness, fever and cough. On examination there was no hepatosplenomegaly. Total count was 4000/cumm, hemoglobin was 4.3gm%, mean corpuscular volume was 105 fl, and platelet count was 16,000/cumm. Peripheral blood smear erythrocytes showing macrocytosis, white blood cells showed 20% blasts which were large and monocytoid with auer rods which were round to oval with raised N:C ratio, disperse chromatin and one to two prominent nucleoli , platelet counts were decreased. Bone marrow aspiration revealed hyper cellular marrow with M: E ratio -5:1.Erythroid series showed megaloblastic maturation with few binucleate forms, myeloid series were also reduced in number with presence of immature cells (blasts) having high N: C ratio, coarse chromatin and 1-2 prominent nucleoli. Blasts constituted more than 25%.Megakaryocytes were reduced in number.

PANCYTOPENIA WITH PURE RED CELL APLASIA

In the present study, one of the case of pure red cell aplasia was observed which presented with pancytopenia. A 62 year old female who presented with fever and generalized weakness, on examination showed pallor, with no signs of lymphadenopathy or hepatosplenomegaly. Total

leucocyte count was 4000/cumm, Hemoglobin was 3 gm/dl, mean corpuscular volume was 51fl and platelet count was 28,000/cumm. Peripheral blood smear showed microcytic hypochromic blood picture. Bone marrow aspiration revealed hypocellular bone marrow and M: E ratio was found to be 2:1. Erythroid series was reduced showing few micronormoblastic maturation and also seen were bizarre (Red Blood Cell) RBC lineage, Myeloid series showed dysplastic changes and showed increased mast cells. Megakaryocytes were normal in morphology. Hence, the diagnosis of pure red cell aplasia was made based on the bone marrow aspiration findings observed.

PANCYTOPENIA WITH HERIDITARY ELLIPTOCYTOSIS

In the present study, one case of hereditary elliptocytosis was observed. A fifty year old male with family history of hereditary elliptocytosis presented with fever, cold, bodyache and malena. Total count was 3,800/cumm, hemoglobin was 9 gm/dl, mean corpuscular volume was 92 fl, and platelet count was 60,000/cumm. Peripheral blood smear showed severe anisopoikilocytosis with cigar shaped red blood cells – Elliptocytosis, white blood cells were normal in morphology, No atypical cells were obsereved in the smear studied. Bone marrow was hypocellular with M: E ratio was 1:2. Erythroid series showed erythroid hyperplasia with normoblastic maturation, myeloid series and megakaryocytes were normal in number and morphology.

STATISTICAL ANALYSIS

Continuous data was summarized in percentage. The categorical variables were compared by chi-square test. Pearson's correlation analysis was used to asses association between the variables. A p value <0.05 was considered statistically significant.

Table: 33 Correlation of age with bone marrow findings

			Megaloblastic anemia	Nutritional anemia	Others	Total	Chi square value(df=10)	P VALUE
Age	< 20 years	Count	7	2	0	9	14.519	0.151
		%	19.4%	7.7%	0.0%	12.9%		
	21 to 30 years	Count	8	5	1	14		
		%	22.2%	19.2%	12.5%	20.0%		
	31 to 40 years	Count	4	6	2	12		
		%	11.1%	23.1%	25.0%	17.1%		
	41 to 50 years	Count	4	5	0	9		
		%	11.1%	19.2%	0.0%	12.9%		
	51 to 60 years	Count	10	2	4	16		
		%	27.8%	7.7%	50.0%	22.9%		
	> 60 years	Count	3	6	1	10		
		%	8.3%	23.1%	12.5%	14.3%		
Total		Count	36	26	8	70		
		%	100.0%	100.0%	100.0%	100.0%		

The difference observed in the bone marrow findings with respect to age was statistically insignificant (Value = 14.15, p = 0.151).

Table: 34 Correlation of sex with bone marrow findings

			Megaloblastic anemia	Nutritional anemia	Others	Total	Chi square value(df=2)	P value
SEX	Male	Count	16	12	3	31	7.981	0.18
		%	44.4%	46.1%	37.5%	44.3%		
	Female	Count	20	14	5	39		
		%	55.6%	53.8%	62.5%	55.7%		
Total		Count	36	26	8	70		
		%	100.0%	100.0%	100.0%	100.0%		

The difference observed in the bone marrow findings with respect to sex was statistically significant (Value = 7.981, p =0.18).

Table: 35 Correlation of total leucocyte count with bone marrow findings

			Megaloblastic anemia	Nutritional anemia	Others	Total	Chi square value(df=6)	P value
TLC	0.4 to 1	Count	0	1	2	3	13.836	0.032
		%	0.0%	3.8%	25.0%	4.3%		
	1.1 to 2	Count	6	2	1	9		
		%	16.7%	7.7%	12.5%	12.9%		
	2.1 to 3	Count	13	8	0	21		
		%	36.1%	30.8%	0.0%	30.0%		
	3.1 to 4	Count	17	15	5	37		
		%	47.2%	57.7%	62.5%	52.9%		
Total		Count	36	26	8	70		
		%	100.0%	100.0%	100.0%	100.0%		

The difference observed in bone marrow findings with respect to total leucocyte count was statistically significant (Value = 13.836, p = 0.032).

Table: 36 Correlation of hemoglobin levels with bone marrow findings

			Megaloblastic anemia	Nutritional anemia	Others	Total	Chi square value(df=6)	P value
HB	1 to 3	Count	5	5	0	10	7.407	0.285
		%	13.9%	19.2%	0.0%	14.3%		
	3.1 to 5	Count	14	8	2	24		
		%	38.9%	30.8%	25.0%	34.3%		
	5.1 to 7	Count	9	2	2	13		
		%	25.0%	7.7%	25.0%	18.6%		
	7.1 to 10	Count	8	11	4	23		
		%	22.2%	42.3%	50.0%	32.9%		
Total		Count	36	26	8	70		
		%	100.0%	100.0%	100.0%	100.0%		

The difference observed in the bone marrow findings with respect to hemoglobin was statistically insignificant (Value= 7.407, p = 0.285).

Table: 37 Correlation of MCV with bone marrow findings

			Megaloblastic anemia	Nutritional anemia	Others	Total	Chi square value(df=10)	P value
MCV	50 to 70	Count	2	5	0	7	22.070	0.015
		%	5.6%	19.2%	0.0%	10.0%		
	71 to 80	Count	2	7	4	13		
		%	5.6%	26.9%	50.0%	18.6%		
	81 to 90	Count	9	9	1	19		
		%	25.0%	34.6%	12.5%	27.1%		
	91 to 100	Count	12	1	2	15		
		%	33.3%	3.8%	25.0%	21.4%		
	101 to 110	Count	7	3	1	11		
		%	19.4%	11.5%	12.5%	15.7%		
	111 to 120	Count	4	1	0	5		
		%	11.1%	3.8%	0.0%	7.1%		
Total		Count	36	26	8	70		
		%	100.0%	100.0%	100.0%	100.0%		

The difference observed in the bone marrow findings with respect to Mean corpuscular volume (MCV) was statistically significant (Value = 22.070, p = 0.015).

Table: 38 Correlation of platelet count with bone marrow findings

			Megaloblastic anemia	Nutritional anemia	Others	Total	Chi square value (df=6)	P value
PLT	< 24000	Count	9	6	3	18	4.909	0.556
		%	25.0%	23.1%	37.5%	25.7%		
	25000 to 50000	Count	10	5	3	18		
		%	27.8%	19.2%	37.5%	25.7%		
	51000 to 75000	Count	4	7	1	12		
		%	11.1%	26.9%	12.5%	17.1%		
	760000 to 100000	Count	13	8	1	22		
		%	36.1%	30.8%	12.5%	31.4%		
Total		Count	36	26	8	70		
		%	100.0%	100.0%	100.0%	100.0%		

The difference observed in the bone marrow findings with respect to platelet count was statistically insignificant. (Value = 4.909, p = 0.556).

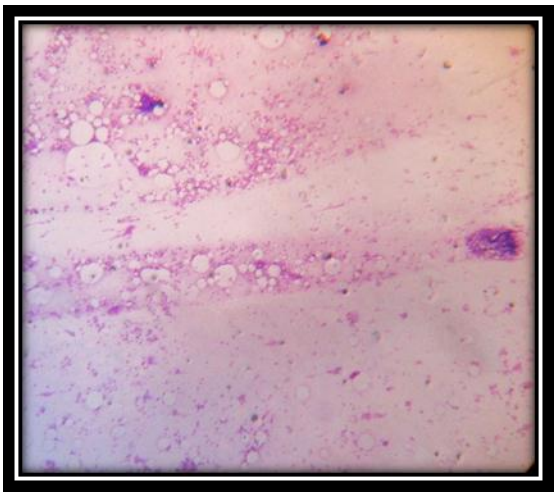


Figure: 8c Hypocellular marrow. Leishman stain (10X)

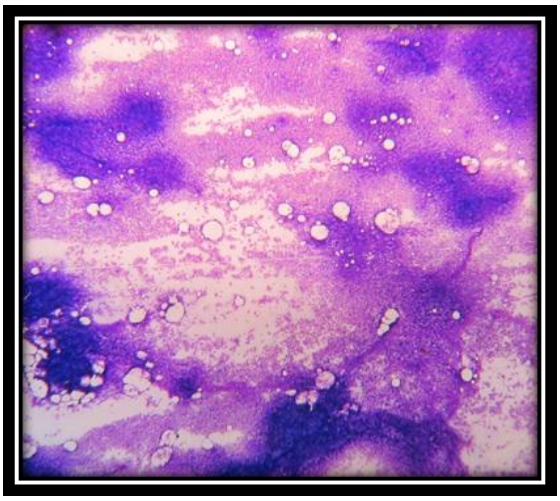


Figure: 8d Hypercellular marrow Geimsa (10X)

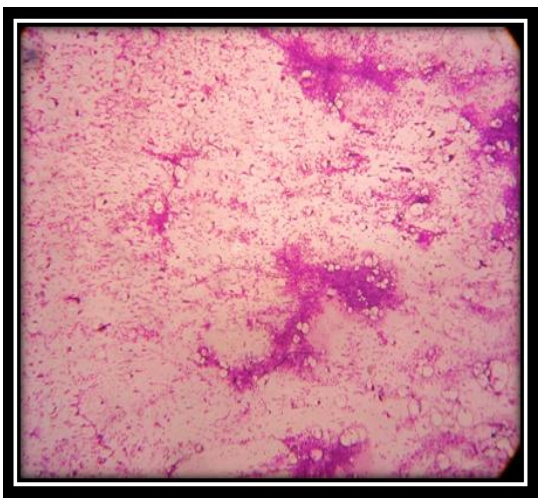


Figure: 8e Normocellular marrow Geimsa (10X)

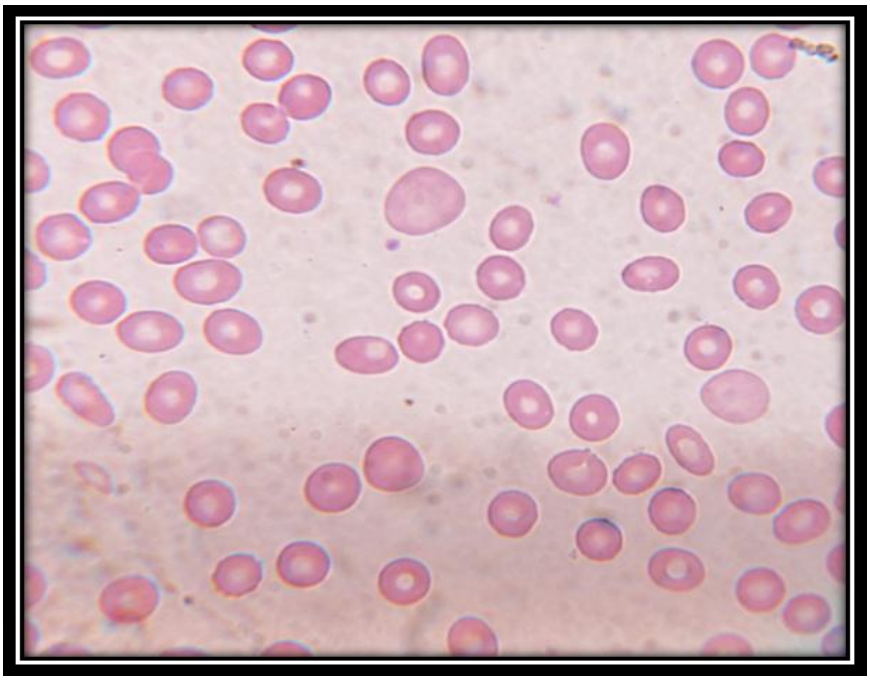


Figure: 32 Anisocytosis with Macro-ovalocytes. Leishman (100x)

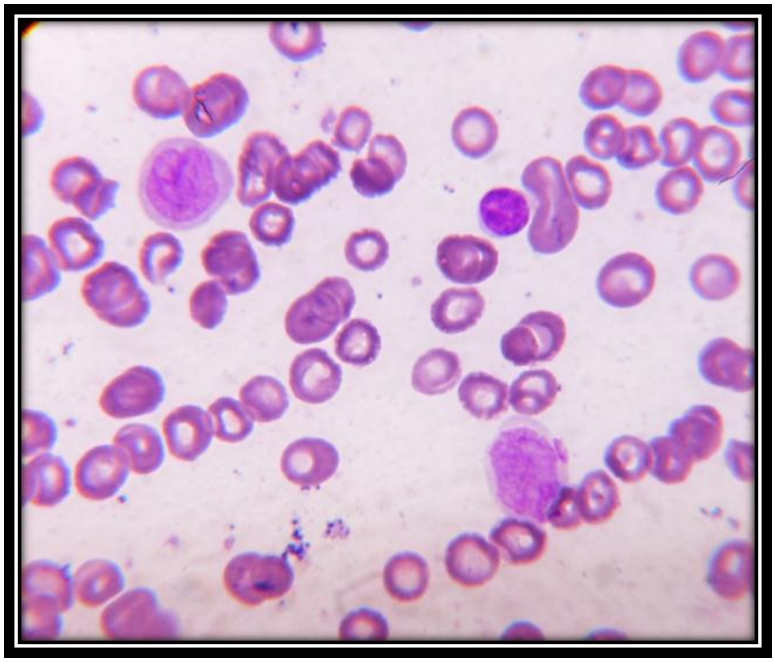


Figure: 33a Monocytoid blast cells with Auer rods-(AML M4).Leishaman stain (100X)

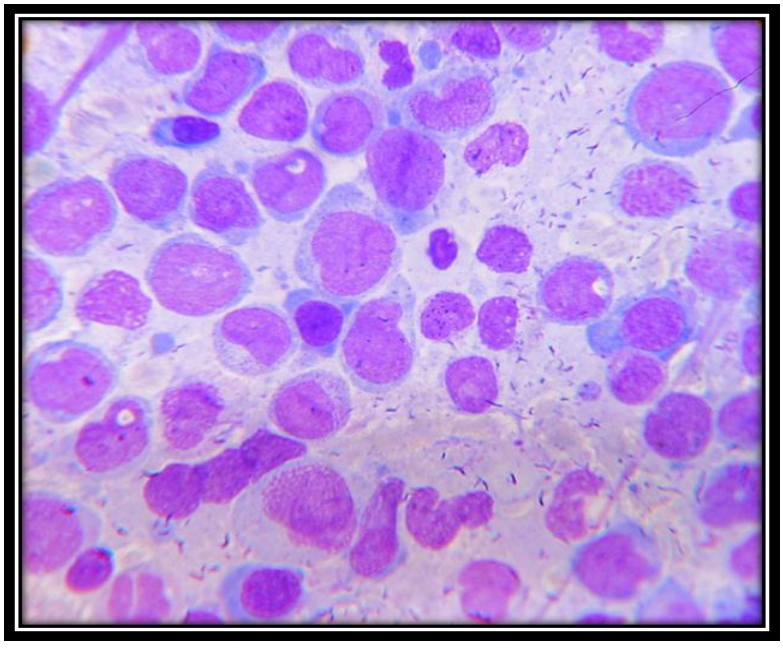


Figure: 33b Myeloblasts with prominent nucleoli in bone marrow.Geimsa (100X)

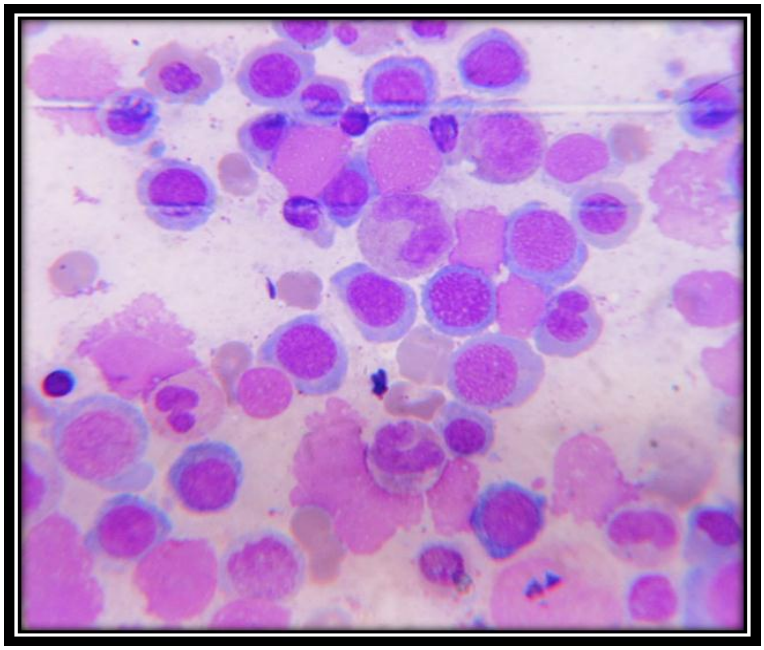


Figure: 34 Megaloblasts with Sieve like chromatin.Geimsa stain (100X)

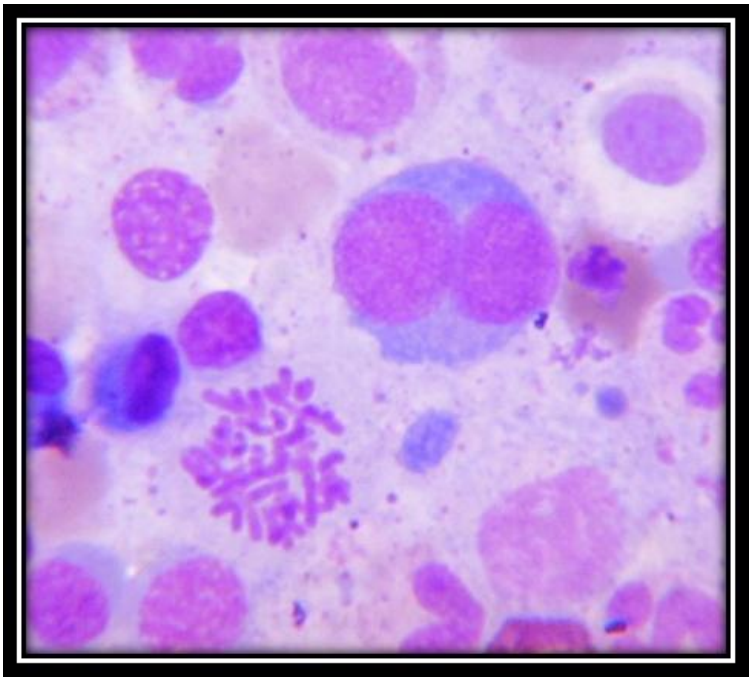


Figure: 35a Dyserythropoiesis- Binucleate forms.Geimsa stain (100X)

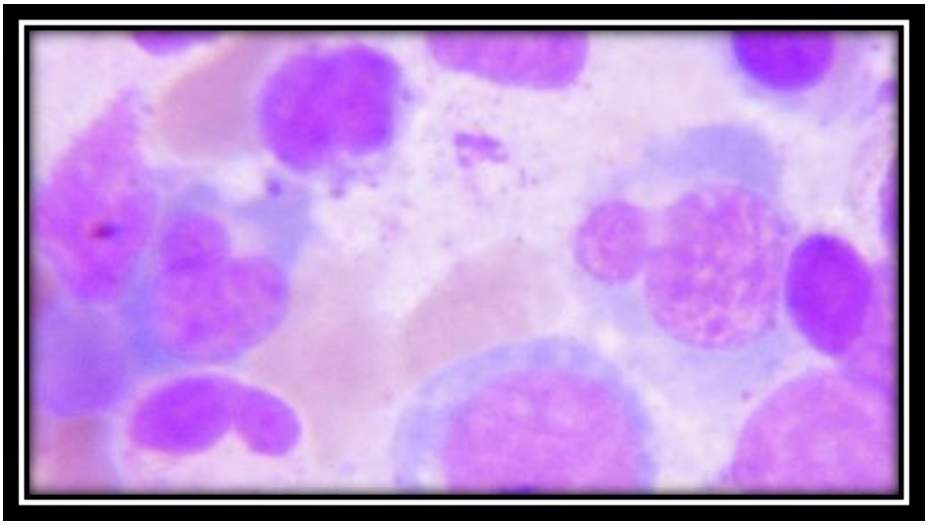


Figure: 35b Dyserythropoiesis – Budding forms.Geimsa stain (100X)

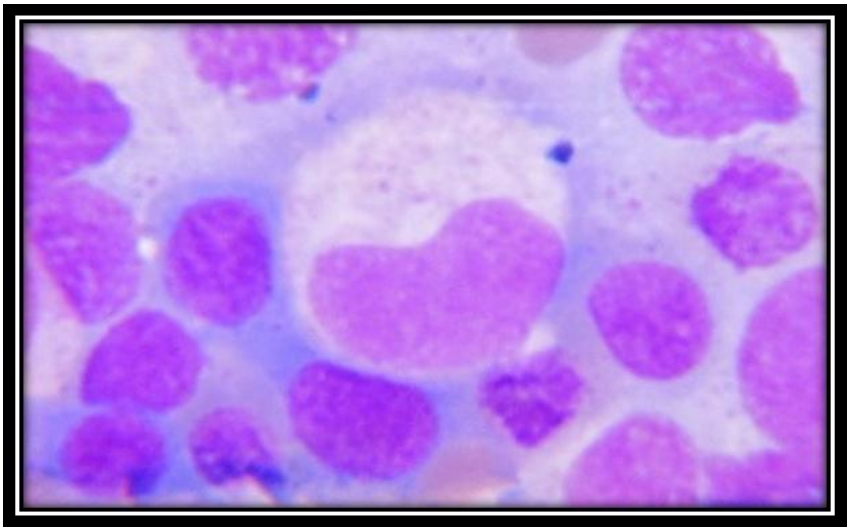


Figure: 36 Giant Metamyelocytes in bone marrow. Geimsa Stain (100X)

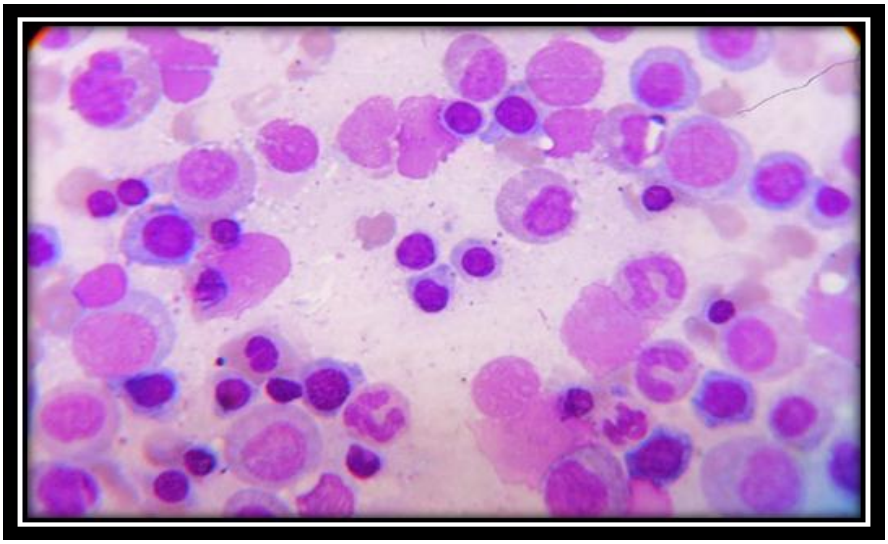


Figure: 37 Micronormoblastic maturation in Bone Marrow. Geimsa Stain (100X)

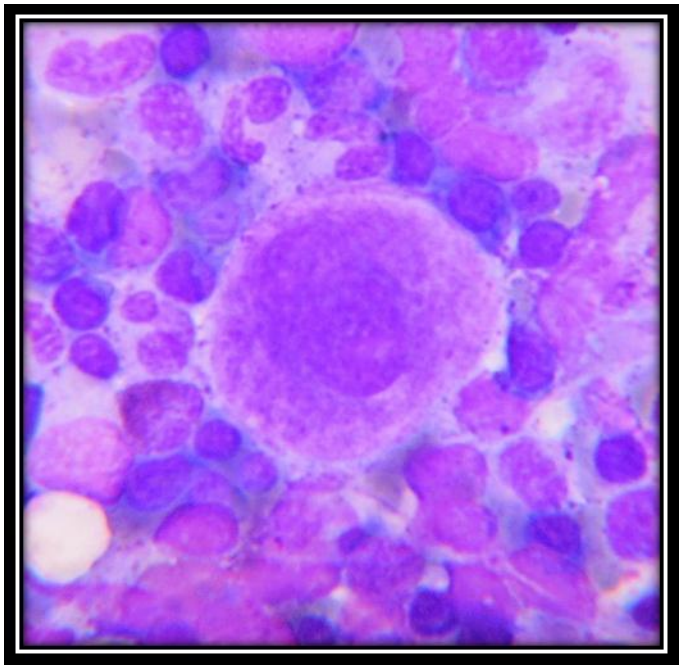


Figure: 38 Hypolobated Megakaryocyte. Geimsa Stain (100X)

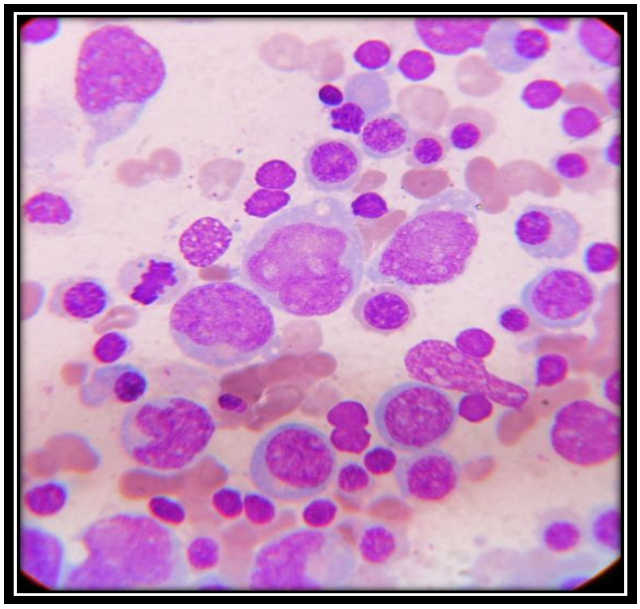


Figure: 39a MDS Bone marrow- myeloblasts. Geimsa stain (100X)

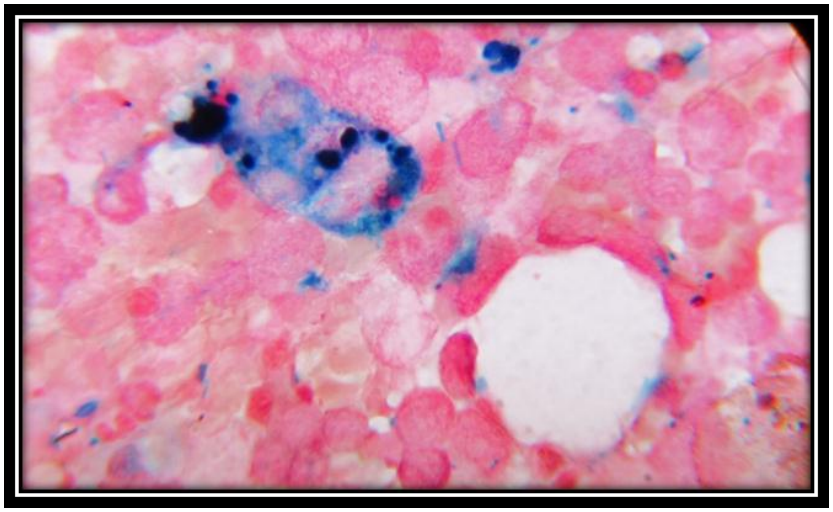


Figure: 39b MDS – Ringed sideroblasts in Bone Marrow. Geimsa stain (100X)

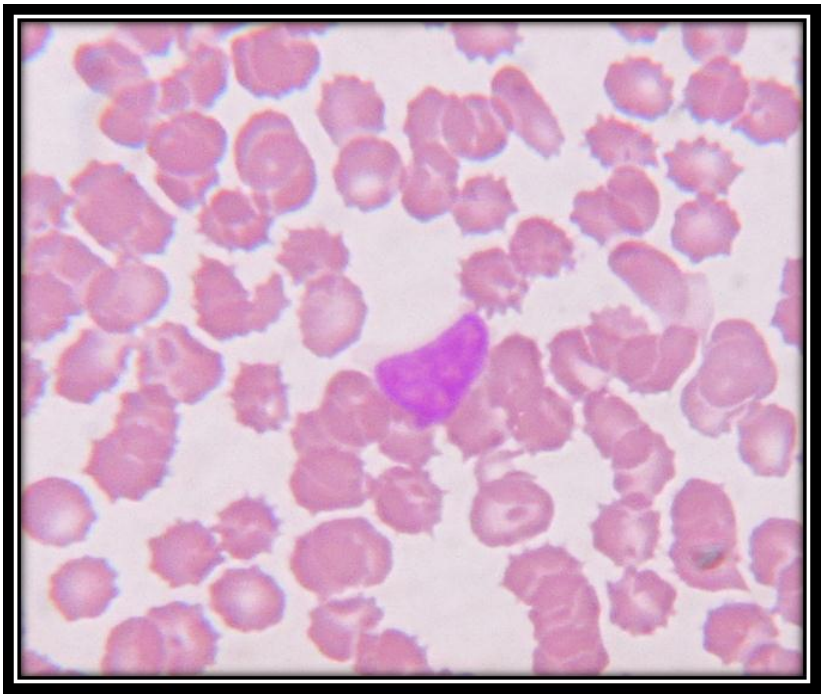


Figure: 40 PBS showing atypical lymphocyte.Leishaman stain (100X)

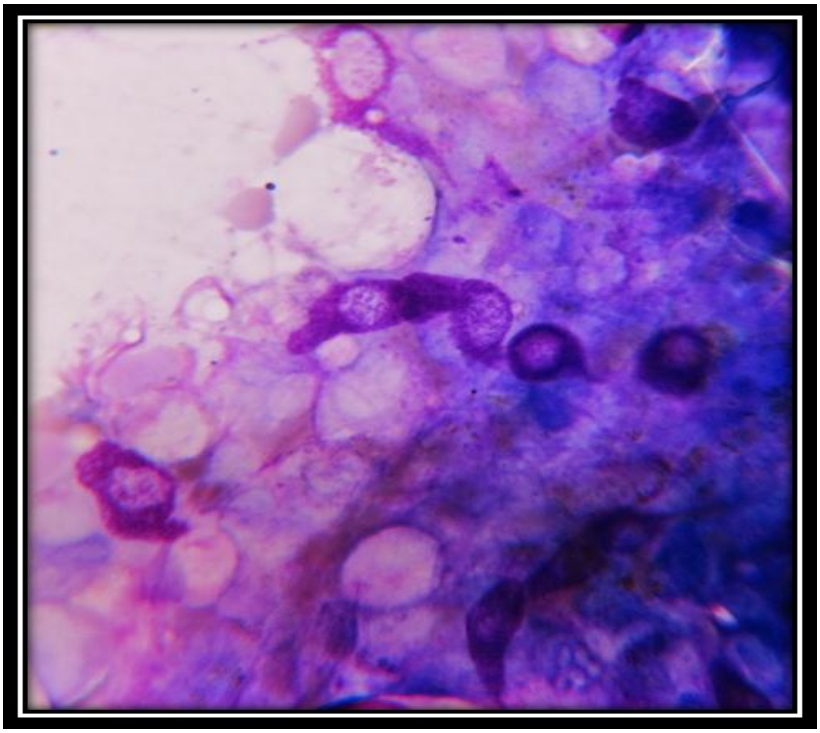


Figure: 41 PRCA – Bizarre RBC Lineage: Giant Proerythroblasts. Leishman stain (40X)

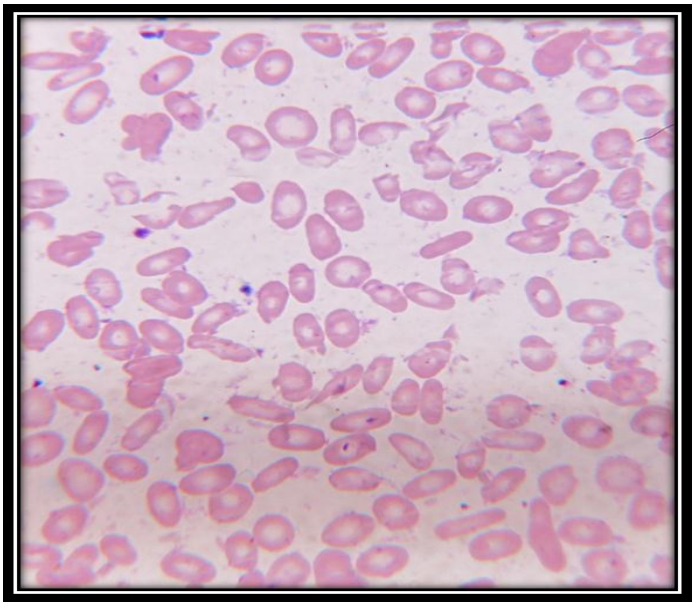


Figure: 42 ELLIPTOCYTES – Cigar shaped RBC's. Leishman stain (100X)

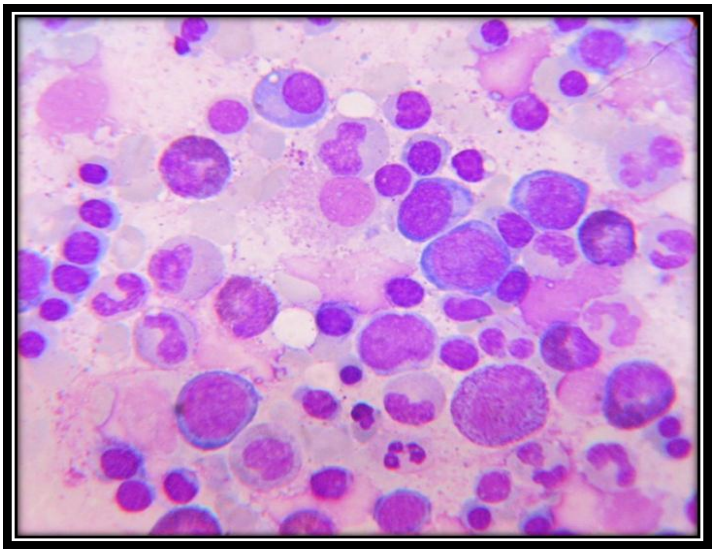


Figure: 43 Erythroid hyperplasia with normoblastic maturation .Geimsa Stain (100X)

DISCUSSION

The variation in the frequency of various diagnostic entities causing pancytopenia in different population groups has been attributed to differences in methodology and stringency of diagnostic criteria, period of observation, geographic area, age pattern, nutritional status, and prevalence of infective disorders, genetic differences, and varying exposure to myelotoxic agents amongst other.

A detailed clinical background and hematological investigation forms the back bone in determining the causes of pancytopenia.

Inspite of its invasive nature, bone marrow examination aids in predicting the outcome of pancytopenia patients, whether it is bone marrow aspiration or biopsy, it is considered as one of the most frequent and safest procedure done routinely in clinical practice. It can be performed easily in the presence of severe thrombocytopenia with little or no risk of bleeding.[68] Bone marrow examination is commonly done for diagnosing unexplained cytopenias, staging of a neoplasm and storage disorders. Trephine biopsy is usually performed in case of hypoplasia or aplasia or dry aspiration, it is also done in cases of lymphomas, granulomatous conditions and malignancies.[69]

In the present study, out of 70 patients, bone marrow aspiration was performed in 67 cases which yielded adequate marrow material. In three cases bone marrow biopsy was performed, as bone marrow aspiration was unsuccessful in these cases. Hence, even when aspiration is unsuccessful biopsy can be used as an adjunct procedure. In the present study, megaloblastic anemia 36(51.4%) was the commonest cause of pancytopenia followed by mixed nutritional

anemia 26(37.1%), viral infections 3(4.2%), MDS (3.8 %), acute leukemia (1.4 %)and one case each of pure red cell aplasia(1.4%) and hereditary elliptocytosis (1.4%).

Sl	Study	Country	Year	No of Cases	Commonest Cause	Second commonest Cause
1	Varma N, Dash S	India	1992	202	Aplastic anemia (40.6%)	Megaloblastic anemia (23.2%)
2	Tilak V,Jain R	India	1998	77	Megaloblastic anemia (68%)	Aplastic anemia (7.7%)
3	Savage D G et al.	Zimbabwe	1999	134	Megaloblastic anemia	Aplastic anemia
4	Khodke et al.	India	2000	166	Hypoplastic anemia (29.5%)	Megaloblastic anemia (22.3%)
5	Naeem Khan et al.	Pakistan	2001	30	Aplastic anemia (20%)	Megaloblastic anemia (16.7%)
6	Kumar R et al.	India	2001	166	Aplastic anemia (29.5%)	Megaloblastic anemia (22.3%)
7	Jha et al.	Nepal	2008	148	Hypoplastic anemia (29.5%)	Megaloblastic anemia (23.6%)
8	BNGayathri et al	Karnataka	2011	200	Megaloblastic anemia (74.4%)	Aplastic anemia (18.26%)
9	Santra G et al	Kolkata	2010	111	Idiopathic plastic anemia (20.72 %)	Hypersplenism due to chronic liver disease (11.71 %)
10	Verma Nidhi et al	Meerut	2012	72	Megaloblastic anemia (40.3%)	Aplastic anemia (22.2%)
11	Taj Ali Khan et al	Peshawar	2013	160	Aplastic marrow (37.5%)	Megaloblastic anemia (13.5%)
12	Arvind Jain et al	Maharashtra	2013	250	Hypersplenism (29.2%)	Infections (25.6%)
13	Baghwan Singh Et al	Indore	2014	53	Megaloblastic anemia (35.4%)	Septicemia (11.32%)
14	DT Sachin et al	Nagpur	2014	210	Megaloblastic anemia (65.71%)	Aplastic anemia (22.38%)
15	Shailaja Prabhala et al	Telangana	2014	172	Megaloblastic anemia (38.95%)	Erythroid hyperplasia (19.18 %)
16	M A Azad et al	China	2015	25	Megaloblastic Anemia (28%)	Aplastic Anemia (20%)
17	Present study	Kolar	2015	70	Megaloblastic anemia (51.4%)	Nutritional anemia (37.1%)

Table: 39 Comparison of causes of pancytopenia with other studies

Verma N et al found aplastic anemia in 40.6% and megaloblastic anemia in 23.26% of patients, [70] as shown in table: 39

Tilak V, Jain R found megaloblastic anemia (68%) to be the commonest cause of pancytopenia followed by aplastic anemia (7.7%).[5] Kumar et al. found hypoplastic anemia (29.5%) to be the commonest cause followed by megaloblastic anemia.[71] Savage D G et al. found megaloblastic anemia to be the commonest cause followed by aplastic anemia[72] as shown in Table:39

Jha et al found Hypoplastic anemia in 29.5 % and Megaloblastic anemia 23.6% of the patients.[3] B N. Gayathri, Rao KS found that the commonest cause of pancytopenia is megaloblastic anemia 74.4% followed by Aplastic anemia was seen in 18.26%, and concluded that a detailed primary hematological investigation with bone marrow aspiration in cytopenic patients are useful to diagnose or rule out the causes of cytopenia.[83]

DT Sachin et al showed that megaloblastic anemia seen in 65.7% was the commonest cause of pancytopenia followed by aplastic anemia seen in 22.3% of the cases, acute lymphoblastic leukemia, acute myeloid leukemia, Gauchers disease, gelatinous bone marrow transformation, myelodysplastic syndrome, deposits of epithelial malignancy, granulomatous disease, multiple myeloma.[73] P Shailaja et al showed that megaloblastic anemia in 38.9% of the patients was the commonest cause of pancytopenia.[74] It was also observed that 7 patients who were on anti-tubercular treatment showed pancytopenia as anti-tubercular drugs are known to reduce serum folate levels in megaloblastic anemia.

M A Azad et al showed megaloblastic Anemia in 28% of patients was the commonest cause of pancytopenia followed by aplastic Anemia seen in 20% of the patients.[75] The other causes included Myelodysplastic syndrome, acute myeloid leukemia, Non-Hodgkin's lymphoma, and

SLE, HIV/AIDS, Hypersplenism and Hemophagocytic syndrome. The commonest cause of pancytopenia, reported from various studies throughout the world has been aplastic anemia followed by megaloblastic anemia as shown in Table: 39

This is in sharp contrast with the results of the present study where the commonest cause of pancytopenia was megaloblastic anemia followed by nutritional anemia. This thus reflects the higher prevalence of mixed nutritional anemia in our population. In the present study, megaloblastic anemia was obsereved 36(51.4%) and the second most common cause was found to be nutritional anemia seen in 26(37.1%).The possible explanation for this would be due to low socioeconomic status, food habits, superstitious beliefs and due to lower literacy rates patients are less compliant towards treatment.

Table: 40 Comparison of cases in nutritional anemia

PARAMETER COMPARED	**Mobina et al[76]**	**Shazia Menon et al[77]**	**Present study**
Percentage of cases	11.2%	8.7%	27.2%

Mixed nutritional anemia is well recognised as a common etiological factor causing pancytopenia. Iron deficiency anemia also results as a nutritional deficiency. Hence, mixed deficiency anemias due to deficiency of iron, vitamin B12 and folate can cause peripheral cytopenia.

In the study done by Mobina et al on 392 cases of pancytopenia, (11.2 %) were found to be due to mixed deficiency anemias.[76] in the study done by Shazia Menon, mixed deficiency was seen in 20 cases (8.69%).[77] Table: 40

In the present study mixed deficiency was seen in 26(37.1%) patients as shown in the above table: 40, the possible explanation may be that, majority of the cases present with anemia rather than pancytopenia and are diagnosed on peripheral smear examination and treated as outpatients. This percentage is much lower than the expected because 60-80% of world population is affected by iron deficiency anemia which is the most common preventable nutritional deficiency in the world.Table:40

Vitamin B12, folic acid and iron studies were not done as it was not affordable by the patients.

Table: 41 Comparison of age and sex distribution in pancytopenia

STUDY	**YEAR**	**MAXIMUM AGE RANGE(in years)**
Jain et al	2013	31-40
Kirpal Das Makheja et al	2013	41-50
Shailaja Prabhala et al	2014	21-30
M. A. Azaad et al	2015	30-59
Senjuthi Dasgupta	2015	5-15
Present study	2015	51- 60

In a study done by Kripal Das Makheja et al megaloblastic anemia was seen in the age group of 41-50 years and had a male to female ratio of 1.2:1.[78] In a study done by Senjuthi Dasgupta et al the common age group was 5-15 years the male to female ratio was 1.7:1.[79]

In the present study, age ranged from 51-60 years, which was found to have a statistically insignificant association (p=0.151); table: 42 and the female to male ratio is 1.2:1, with a female preponderance in megaloblastic anemia; which was had a statistically significant association

(p=0.018). This is due to lack of availability of care, nutritional education in the elderly age group. Female are more affected due to social neglect and lack of education, Table: 41

The commonest affected age group in mixed nutritional anemia was more than 60 years. There was a female preponderance and female to male ratio was 2.25:1.Majority of the patients presented with fever, generalised weakness and bleeding tendencies. Pallor was the most common sign seen in 30 (42.8%) of the cases among this study group. This is due to loss of blood through menstruation in addition to nutritional deficiencies.

Table: 42 Comparison of age and sex distribution in malignant diseases

Parameter compared	**Khodke et al[8]**	**Gayathri et al[83]**	**Tilak et al[5]**	**Jha et al[3]**	**Present study**
Age range (years)	3-69	41	5-70	2-75	24

Khodke et al. and Tilak et al. reported one case of AML causing pancytopenia.[5, 8] the age ranged from 3-69 years and 5-70 years respectively. In the study done by Jha et al., acute leukemia alone constituted (90.6%) of all the hematological malignancies. It accounted for (19.5%) of total cases of pancytopenia. Age ranged from 2-75 years with a male to female ratio of 1.9:1.[74]

In the present study, the malignant diseases accounted for 1.4% of pancytopenia. One case of acute myeloid leukemia (AML M4) was encountered which was of 24 year old male presenting with peripheral cytopenia,Table: 42

MYELODYSPLASTIC SYNDROME

Pancytopenia is a common finding in Myelodysplastic syndrome, but least common finding encountered in patients with MDS as compared to mono and bicytopenia. In a study of 31 patients of MDS by Kini J et al., bicytopenia was the commonest finding.[67] Greenberg et al studied 816 patients and observed pancytopenia in 15% of the patients.[56]

Table: 43 Comparison of age and sex distribution in MDS

Parameter Compared	Kini J et al[56]	Juneja SK et al[80]	Present study
Age range (years)	40-70	48-95	40-60

Kini J et al observed that 31patients in the study population were in the age group 40-70 years.[56] in a study of 118 patients with MDS by Juneja SK et al the age ranged from 48-95 years,[80] Table: 43

In the present study, the age group in MDS patients was observed to be between 40 -60 years.

DISTRIBUTION OF SYMPTOMS AND SIGNS IN PANCYTOPENIA

Studies done by B Deepak et al suggests that generalized weakness was the commonest symptom and pallor was the most predominant sign in megaloblastic anemia.[96] Taj ali khan et al in observed that the common clinical presentations were pallor, fever weakness, bleeding manifestations splenomegaly and gastrointestinal symptoms.[81]

In the present study,27(38.5%) of the cases showed easy fatigubility as the commonest presenting symptom in megaloblastic anemia followed by fever and generalised weakness, and

pallor was the most commonest sign observed seen in 32(45.7%) of the cases followed by splenomegaly and hepatomegaly.

Patients with mixed nutritional anemia presented with fever, generalised weakness and bleeding tendencies .Pallor was the most common sign seen in 30 (42.8%) of the cases among this study group attributable to low hemoglobin levels.

Table: 44 Comparison of hematological parameters in pancytopenia

STUDY	YEAR	HEMATOLOGICAL PARAMETERS		
		HB(in gm/dl)	TLC(in cu/mm)	PLATELET(in cu/mm)
Kumar et al.	2001	2.4-7	700-3.600	10.000-1.3 L
Jha et al	2008	2.3-9.8	1,200-3.900	2,000-1.37L
B N Gayathri et al	2011	1.9-2.9	500-3,900	12,000-95,000
M. A. Azad et al	2015	6.6-7.6	2,000-3,000	25,000-50,000
Senjuthi Dasgupta	2015	2.3-9	700-3,500	11,000-95,000
Present study	2015	3.1-5	3,100-4,000	25,000-50,000

In the study of pancytopenia cases by Kumar et al., hemoglobin ranged from 2.4-7 gm/dl, TLC ranged from 700-3600 cells/mm and platelet count ranged from 1,00,000-1,30,000 cells/mm.[84] In the study done by Jha et al, hemoglobin ranged from 2.3-9.8 gm/dl, TLC ranged from 1200-3900 cells/mm3 and platelet count ranged from 2000-1,37,000 cells/mm.[86] In a study done by B N Gayathri et al hemoglobin ranged from1.9-2.9 gm/dl, TLC ranged from1200-3900 cells/mm and platelet count ranged from 12,000-95,000 cells/mm.[98] In a another study done by Senjuthi Dasgupta et al, hemoglobin ranged from 2.3-9 gm/dl, TLC ranged from 700-3500 cells/mm and platelet count ranged from 11000-95000 cells/mm.[79]

In the present study, hemoglobin ranged from 3.1-5 gm/dl; which has an insignificant statistical association (p=0.285), TLC ranged from 3100-4000 cells/cumm; which had a statistically significant association (p=0.032) and platelet count ranged from 76,000-1, 00,000 cells/cum; which had a statistically insignificant association (p=0.556), Table: 44

MEGALOBLASTIC ANEMIA

In megaloblastic anemia the maximum hemoglobin range was 3.1-5 gm/dl seen in 18(25.7%) of the cases, total leucocyte count was between 3100-4000/cumm seen in 17(24.2%) of cases.MCV levels of 91-100 fl was 11(15.7%) statistically significant association (p=0.015). Platelet count range was 76000-1, 00,000/cumm in 14(20%) of the patients.

MIXED NUTRITIONAL ANEMIA

The maximum hemoglobin range in mixed nutritional anemia was from 1-3 gm/dl, which was seen in 11(15.7%) of the cases. Total leucocyte count range was between 3100-4000/cumm seen in 15(21.4%) of the cases and the maximum range of Mean corpuscular volume was between 81-90 fl seen in 9(12.8%) of the cases. The platelet count ranged from 76,000 to one lakh/cumm seen in 7(10%) of the cases.

Table: 45 Comparison of hematological parameters in malignant diseases

Parameters compared	**Kumar et al[71]**	**Jha et al[3]**	**Present study**
Hb (gm/dl)	2.6 - 6	1.2 - 9.6	4.1-5
TLC(cells/mm3)	650-3200	800-3600	4000
Platelet(cells/mm3)	18000 - 96000	6000-1.25L	16,000

In the study done by Kumar et al., hemoglobin ranged from 2.6-6 gm/dl, TLC ranged from 650-3200 cells/cumm and platelet count ranged from 18,000-96,000 cells/cumm.[71] In the study done

by Jha et al, hemoglobin ranged from 1.2-9.6 gm/dl, TLC ranged from 800-3600 cells/cumm and platelet count ranged from 6,000-1,25,000 cells/cumm.[3]

In the present study, hemoglobin was from 4.3 gm/dl, TLC was 4000 cells/cumm and platelet count was 16,000 cells/cumm, Table: 45

PERIPHERAL BLOOD SMEAR

MEGALOBLASTIC ANEMIA

Khodke K et al observed that 20 out of 22 cases showed anisocytosis, 10 out of 22 cases showed dimorphic blood picture.[8] In a study done by B Deepak et al, anisocytosis was the most important peripheral blood finding in megaloblastic anemia.[81] DT Sachin et al observed that macrocytic anemia was the predominant blood picture.[73]

In the present study, peripheral smear in megaloblastic anemia showed macrovalocytes with moderate degree of anisopoikilocytosis, as shown in Fig: 32 with dimorphic anemia in 50% of cases were observed in which was consistent with other studies.

MIXED NUTRITIONAL ANEMIA

The peripheral blood smear in mixed nutritional anemia was composed of both macrocytes and microcytes was seen in 10(14.2%) of the cases, 7(10%) of the cases showed normocytic anemia and 7(10%) of the cases showed microcytic hypochromic anemia. This was attributable to the iron deficiency with added on vitamin B 12 and folate deficiency, thus concluding mixed nutritional anemia.

ACUTE LEUKEMIA

Khodke K et al observed one case of acute myeloid leukemia with immature cells in the peripheral blood was reported.[8] In the study done by Tilak et al one case of acute myeloid leukemia with anisocytosis, circulating erythroblasts and immature cells was reported[83]. In the present study, one case of acute myeloid leukemia peripheral blood smear showed macrocytic anemia and 20 % circulating monocytoid blasts with Auer rods as shown in Fig:33a and was categorized as AML-M4.Platelet count was also decreased. Hence, careful morphological evaluation of white blood cells has to be done in pancytopenia patients.

MYELODYSPLASTIC SYNDROME

MDS is characterized by peripheral pancytopenia despite a normocellular or hypercellular bone marrow because of increased apoptosis of hematopoietic bone marrow resulting in ineffective erythropoiesis.[84]

In two cases of MDS peripheral blood smear erythrocytes showed normocytes with decreased white blood cell counts and platelets counts.

BONE MARROW EXAMINATION

MEGALOBLASTIC ANEMIA

In a study done by DT Sachin et al showed that the commonest bone marrow finding was megaloblastic erythropoiesis.[73]

In the present study, bone marrow was hypercellular in 20 (64.5%) of patients and normocellular in 11 (43.2%) of the cases. The bone marrow picture in megaloblastic anemia was predominantly megaloblastic maturation showing megaloblasts showing nuclear cytoplasmic asynchrony as

shown in Figure: 34 with pale blue cytoplasm and large nucleus with sieve like chromatin accounting to megaloblastic anemia being one of the commonest cause of pancytopenia attributable to vitamin B 12 and folate deficiency. This cause of cellular gigantism is an impairment in Deoxyribonucleic acid (DNA) synthesis which delays nuclear maturation and cell division, because RNA and cytoplasmic elements are synthesized at a constant rate despite the cell's impaired DNA synthesis and the cells show nuclear-cytoplasmic asynchrony. However, in this study vitamin B12 and Folic acid levels were not assessed. Also 15(21.4%) of the cases showed features of dyserythropoiesis in the form of binucleate forms and budding forms. Figure: 35a and Figure: 35b. In myeloid series giant metamyelocytes were seen in 8(11.4%) of the cases was a significant finding in megaloblastic anemia. Giant metamyelocyte is an atypical myeloid cell with clumped chromatin in a large, often bizarre immature nucleus and relatively mature cytoplasm, as shown in Figure: 36 This is due to Vitamin B12 and folate deficiency in megaloblastic anemia, the nuclei cannot mature adequately. Megakaryocytes showed hypolobated forms and giant forms. Idealy, a normal megakaryocyte has four to sixteen nuclear lobes and hypolobated or immature megakaryocyte is defined as a form having bluish cytoplasm and lacking lobulation of the nucleus which occupies most of the cell.

MIXED NUTRITIONAL ANEMIA

In mixed nutritional anemia, bone marrow showed erythroid hyperplasia with predominantly micronormoblastic maturation with megaloblastic maturation and normoblastic maturation was observed in most of the patients Figure: 37 Leucopoiesis was normal and Megakaryopoiesis was normal with no morphological abnormalities.

ACUTE LEUKEMIA

Bone marrow in this case of acute leukemia showed a hyper cellularity with M: E ratio - 5:1.Erythroid series showed megaloblastic maturation with few binucleate forms, myeloid series were also reduced in number with presence of blasts which constituted more than 25%.Fig:33b Careful assessment should be done and subsequent bone marrow aspiration and biopsy should be asked for in doubtful cases.

MYELODYSPLASTIC SYNDROME

Bone marrow examination revealed hypercellular marrow with raised M: E ratio. Erythroid series showed dyserythropoietic features like nuclear fragmentation, budding multiple nuclei and hyperlobulation. Myeloid series were reduced in number with few atypical cells seen constituting around blasts, pearls stain showed ringed sideroblasts. Fig: 39a, 39b.Megakaryocytes showed hypolobulated forms, as shown in Figure: 38 and dysplastic changes.

Increased apoptosis of hematopoietic bone marrow results in ineffective hematopoiesis. Inhibition of apoptotic mechanism may induce leukemic transformation in MDS.[84]

PANCYTOPENIA WITH VIRAL INFECTIONS

Thisyakorn U et al (1984) studied peripheral smear and buffy coat smear in 40 serological confirmed dengue hemorrhagic fever patients, and in other patients with viral and bacterial infections. Atypical lymphocytosis was found in majority of dengue hemorrhagic fever both associated with secondary and also in dengue fever with primary infection as compared to other infections.[85]

Boonpucknavig S et al studied eighty-eight specimens consisting of lymphocytes separated from peripheral blood samples from 76 patients with dengue hemorrhagic fever (DHF), which revealed a significant increase in numbers of atypical lymphocytes[86]

Jampangern, et al postulated that the atypical lymphocyte is an activated lymphocyte in response to stimulation and expressed activation markers on the cell and also concluded that atypical lymphocyte and CD19+ cell counts are a significant finding, besides platelets and white blood cell counts, as a useful diagnostic tool for dengue infection.[87]

Peripheral blood is the easiest to obtain and readily accessible. A thorough hematological profiling of this constituent from dengue patients has been well documented. The participation from the blood's many individual components, for instance the activation of complement, the status of monocytes and T-lymphocytes and the levels of platelets, is considered to be a good prognostic tool to evaluate the stage of disease and severity of conditions. Many significant findings have been recorded such as the presence of pan-leucopenia and thrombocytopenia and cells with an abnormal morphology such as atypical lymphocytes and an altered ratio of immune cells.[88]

Jih-Jin et al concluded that bone marrow suppression is one of the earliest changes in dengue patients hence investigation into the bone marrow cellularity is vital.[88] Srichaikul et al (2008) showed bone marrow associated aplasia in dengue patients.[89] Consequently, the status of the BM involvement in dengue pathogenesis and the mechanisms leading to its suppression in patients is still unresolved. Hence, the available literature suggests that bone marrow is involved at early stages of infection.. Bone marrow taken at the onset of fever may reveal abnormal bone marrow cellularity (reduction in the levels of megakaryopoiesis, erythropoiesis and granulocytosis) and hypercellularity or normocellularity may be observed when taken at the time

of severe clinical manifestations. Timing is one of the key factors that has to be considered to obtain accurate information about the involvement of the bone marrow in dengue virus infection, and other viral infections as well.[89, 90] an unusual observation of phagocytic reticulum cells has been detected in bone marrow at late stages of infection.[90]

In the present study, there were three cases were categorized as viral infections. All of these three cases showed atypical lymphocytes on peripheral blood smear examination. Figure: 40 However, bone marrow revealed a normocellular marrow with predominantly normoblastic maturation. Myeloid series was normal in morphology and megakaryocytes were normal in number and morphology.

PANCYTOPENIA WITH PURE RED CELL APLASIA

Acquired pure red cell aplasia (PRCA), a part of a unique form of Aplastic Anemia, is a rare condition of profound anemia characterized by the absence of reticulocytes and the virtual absence of erythroid precursors in the bone marrow.

Kaznelson et al (1992) recognized that this condition was a different entity from aplastic anemia, which presents with pancytopenia.[91] the characteristics of PRCA include a severe anemia, a reticulocyte count of less than 1%, and the presence of less than 0.5% mature erythroblasts in the bone marrow. The bone marrow is usually normocellular.

In some cases a bone marrow aspiration typically shows erythroid hypoplasia or aplasia and characteristic large proerythroblasts confirming the diagnosis. If bone marrow is done during the recovery phase it may show active erythropoiesis that can be misleading.

In the present study, one of the case of pure red cell aplasia was observed which presented with pancytopenia. Peripheral blood smear showed microcytic hypochromic blood picture. Bone

marrow aspiration revealed hypocellular bone marrow with reduced erythroid series showing micronormoblastic maturation and bizarre (Red Blood Cell) RBC lineage, Figure: 41 myeloid series showed dysplastic changes and showed increased mast cells. Megakaryocytes were normal in morphology.

PANCYTOPENIA WITH HERIDITARY ELLIPTOCYTOSIS

Transient pure red blood cell aplasia has been reported in patients with chronic hemolysis such as HIM. Under these conditions, the erythrocytes's life span is shortened which inhibits erythropoiesis and cause severe anemia, called as "aplasic crisis".[91]

In the present study, one case of hereditary elliptocytosis was observed in a fifty year old male with family history of hereditary Peripheral blood smear showed severe anisopoikilocytosis with cigar shaped red blood cells – Elliptocytosis; Figure: 42, white blood cells were normal in morphology, No atypical cells were observed in the smear studied. Bone marrow was hypocellular showing erythroid hyperplasia with normoblastic maturation Figure: 43, myeloid series and megakaryocytes were normal in number and morphology.

Several possibilities exist in this case that could explain the pancytopenia. An infectious cause can be agreed upon with a history of a cold, fever and body ache. Other possible causes include drugs which has been reported to cause pancytopenia, but in this case the drug history was insignificant.

CONCLUSION

Megaloblastic anemia was the commonest cause of pancytopenia in the present study. The hematological parameters and bone marrow morphological features in patients with megaloblastic anemia was confirmed by presence of megaloblasts with presence of dyserythropoiesis and giant metamyelocytes. Nutritional anemia was the second most common cause and bone marrow examination revealed predominantly micronormoblastic maturation and megaloblastic maturation indicating mixed deficiencies. The presence of atypical lymphocytes was significant in viral infections. The bone marrow findings in acute leukemia, MDS, pure red cell aplasia and hereditary elliptocytosis aided in the diagnosis and outcome of the patients.

A comprehensive clinical, hematological and bone marrow study of patients with pancytopenia usually helps in the identification of the underlying cause. However, in view of a wide array of etiological factors, pancytopenia continues to be a challenge for the hematologists.

SUMMARY

- This was prospective study on "ETIOPATHOLOGICAL STUDY OF PANCYTOPENIA – AN INSTITUTIONAL STUDY" conducted from 2013 – 2015.Patients diagnosed as a case of pancytopenia having hemoglobin less than 10 gm/dl,Total leucocyte count less than 4000/cumm, Platelets less than 1,00,000/cumm were studied. Seventy patients were selected based on the inclusion and exclusion criteria.
- The objective of the study was to study the clinical presentations and hematological parameters in cases of pancytopenia. Also to study the morphological pattern in bone marrow in patients with pancytopenia
- The most common age group affected by pancytopenia was between 51-60 years. A definite female predominance was observed in our study with 31(44.3%) males and 39(55.7%) females, which had a statistically significant association ($p<0.05$), where the female to male ratio was 1.25:1 in over all the cases of pancytopenia.
- The most common presenting symptom in pancytopenia was fever seen in 45(67.2%) of cases followed by generalised weakness in 37(57.9%), and the most common sign observed was pallor. Hematological data showed 24(34.2%) of the cases showed hemoglobin levels between 3.1-5gm/dl attributable to anemia, 37(55.2%) of the cases showed leucocyte count between 3100-4000/cumm which was statistically significant ($p<0.05$), 23(34.3%) of the cases showed platelet count between 76,000-1, 00,000/cumm and 33(47.2%) of the cases showed mean corpuscular volume (MCV) between 81-90 fl.

However, higher MCV value was a statistically significant entity in megaloblastic anemia. ($p<0.05$)

- The peripheral blood smear showed dimorphic blood picture in 30(42.9%) of the cases of pancytopenia as shown in. On bone marrow examination 34(48.5%) of cases were of hypercellular marrow, 23(32.8%) of cases showed normocellular marrow and 13(18.7%) of the cases showed hypocellular marrow.
- On bone marrow examination of the megaloblastic anemia was one of the commonest cause of pancytopenia in 36(51.4%) with evidence of megaloblastic maturation and dyserythropoiesis (21.4%) attributable to vitamin B 12 and folate deficiency, and the second most common cause was mixed nutritional anemia in 26(37.1%), followed by viral infections in 3(4.2%). Bone marrow examination aided in the diagnosis of MDS, Acute leukemia, pure red cell aplasia. An uncommon presentation of hereditary elliptocytosis was observed, despite the peripheral cytopenia the bone marrow showed normoblastic maturation with myeloid cells and megakaryocytes without morphological abnormalities. Among the myeloid series in pancytopenia, 13(18.6%) of the cases showed abnormal forms like giant metamyelocytes, blast cells and dysplastic changes. Giant metamyelocytes seen in 8(11.4%) of the cases was a significant finding in megaloblastic anemia. The megakaryocytes studied in pancytopenia, 17(24.2%) abnormal forms were observed in the form of hypolobation and giant forms.

Hence, hematological investigations forms the bed rock in the early diagnosis and management in patients with pancytopenia. However, bone marrow examination is a helpful diagnostic tool in predicting the course and outcome of the condition.

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ANNEXURE I

PATIENT PROFORMA

IP NO:

BM NO:

NAME:

AGE:

SEX:

OCCUPATION:

HISTORY OF PRESENTING ILLNESS

COMPLAINTS OF - 1. Fever 2. Bodyache 3. Generalized weakness 4. Respiratory distress 5. Altered sensorium

PAST HISTORY:

DRUG HISTORY:

H/o myelotoxic drugs, h/o chloramphenicol

a)NSAIDS b)Colchichine c)Sulphonamides d)Phenothiazines e) Thiazides f)Anti-thyroid drugs g)Antiepileptics h) Antidiabetics

Vitals

Pulse:

Blood pressure:

GENERAL PHYSICAL EXAMINATION:

PALLOR ICTERUS CLUBBING CYANOSIS EDEMA LYMPHADENOPATHY

SYSTEMIC EXAMINATION:

PER ABDOMEN EXAMINATION: HEPATOMEGALY

SPLEENOMEGALY

CLINICAL DIAGNOSIS:

PERIPHERAL BLOOD SMEAR FINDINGS:

RBC MORPHOLOGY

WBC MORPHOLOGY

PLATELETS COUNT

IMPRESSION:

COMPLETE BLOOD COUNT PARAMETERS:

TOTAL LEUCOCYTE COUNT:

RBC COUNT:

HEMOGLOBIN:

HCT

MCV

MCH

MCHC

PLATELET COUNT

BONE MARROW EXAMINATION:

BIOPSY/ ASPIRATION:

ADEQUACY:

CELLULARITY:

M: E RATIO:

ERYTHROID SERIES:

MYELOID SERIES:

DIFFERENTIAL COUNT:

MEGARYOPOIESIS:

IMPRESSION:

ANNEXURE II

STAINING TECHNIQUE

A. LEISHMAN'S STAIN

1. Smears were first air dried.

2. Then slides were placed on a slide stand and covered with Leishman‟s stain for 5 minutes.

3. Then double the amount of distilled water was added over the Leishman‟s stain and left for 30 minutes.

4. Then smears were washed with distilled water and then air dried.

Results: - Nucleus – Blue; Cytoplasm – Pink

B. PERL's PRUSSIAN BLUE STAIN

Reagents- Fixative (methanol), ferrocyanide reagent solution, acid reagent solution, neutral red solution and distilled water.

Procedure

1. Fix the bone marrow aspirate slide with methanol for 5 minutes.

2. Cover the fixed smear with ferrocyanide and acid reagent solution and wait for 10 minutes.

3. Wash the slides thoroughly with distilled water.

4. Finally counterstain with neutral red solution for 30 seconds only and wash the slide immediately with tap water.

5. Let air dry slide and see under the microscope.

Results: - Iron - blue; Cytoplasm – Red

ANNEXURE III

KEY TO MASTER CHART:

ALT SENSORIUM ALTERED SENSORIUM

AML Acute myeloid leukemia

ATL Atypical Lymphocytes

BA BodyAche

BF Binucleate Forms

BP Body Pain

BL Breathlessness

DA Dimorphic Anemia

Dec Decreased

DG Decreased Granulocytes

DM Dimorphic Maturation

DS Dyspnea

Dys / DYS Dysplasia

E Edema

EH Erythroid hyperplasia

EOSI Eosinophils

F Fever

Fwc Fever with chills

GM Giant Metamyelocytes

GW Generalised Weakness

HE Heriditary Spherocytosis

HM Hepatomegaly

HYPER Hypercellular

HYPO Hypocellular

I Icterus

ImmGranuloct Immature Granulocyte

Inc Increased

L Lymphadenopathy

LA Lower Abdomen Pain

LW Lower limb Weaakness

MA Macrocytic Anemia

MHA Microcytic hypochromic Anemia

MEG Megaloblastic Maturation

MF Multinucleate Forms

MicroM Micronormoblastic Maturation

MIC Micronormoblastic Maturation

MM Megaloblastic Maturation

NA Normocytic Anemia

NB Normoblastic Maturation

NORMO Normocellular

NM Normoblastic Maturation

P Pallor

PA Pain Abdomen

Red Reduced

SL NO	BM NO	IP NO	NAME	AGE	SEX	SYMTOMS	SIGNS	PBS	TLC	Hb	MCV	PLT COUNT	M:E ratio	CELLULARITY	ERYTHROID	MYELOID	MEGAKARYOCYTE	PLASMA/ PARASITES/ GRANULOMAS	BMA/BMB Impression
1	BM-36/13	974911	Chandrakala	34	F	GW,F	P	DA	3.7	6.7	102	30,000	1;1	HYPER	MIC/MEG	GM	HYOLOBATED	NO	EH + DM
2	BM-35/13	972309	Radha	23	F	GW,PA	P	MA	2.7	6.4	102	60,000	1;3	HYPER	MIC/MEG	GM	Giant forms	NO	EH+MM
3	BM-34/13	971922	Naruma	55	F	BP,F	P,E	DA	1.6	4.5	92	92,000	0.7;1	NORMO	MIC/MEG,BF	N	N	NO	EH+DM
4	BM-30/13	950609	Roja	23	F	GW,F	P	DA	3.8	7.8	90	18,000	1;2	HYPER	MEG,BF	N	N	NO	EH+DM
5	BM-27/13	942269	Prasad	52	M	GW,F	P	DA	3.5	5.2	90	60,000	1;1	HYPER	MEG	N	N	NO	EH+MM
6	BM-25/13	938448	Venkatesh.R	19	M	GW	P	DA	2.2	4.8	89	78,000	0.36;1	HYPER	MIC/MEG	N	N	NO	EH+DM
7	BM-20/13	922269	Venkateshappa	40	M	GW,F	P	MA	1.2	7.8	79	87,000	10;1	HYPER	MEG,BF,MF	Myeloblast<9%	Giant,HYPOLOBATED	NO	RAEB-1,myelodysplastic
8	BM-19/13	922735	Sameena Taj	20	F	GW,Fwc	P	NA	4	9.8	80	40,000	2;1	HYPO	MIC	N	N	NO	DM
9	BM-18/13	922260	Venkatareddy	40	M	GW,Fwc	P	NA,ATL	0.4	6.4	76	29,000	2;1	NORMO	NB	N	N	NO	NM
10	BM-17/13	914648	Manjunath	34	M	Fwc	SM,P,I	DA	3.8	9.8	98	50,000	2;1	NORMO	MIC/MEG	N	N	NO	EH+DM
11	BM-11/13	903590	Muniyappa	55	M	GW,Fwc,PA	P	DA	3.9	7.8	79	16,000	1;1	HYPER	MEG	N	HYPOLOBATED	NO	MM
12	BM-10/13	871752	Chikamunivenkatappa	65	M	GW,F	P	NA	3.2	3.8	77	10,000	0.3;1	HYPO	NB	N	N,Dec	NO	Hypoplastic+NM
13	BM-9/13	900213	Krishnappa	55	M	GW,Fwc	P	DA	1.5	2.7	127	33,000	1.4;1	HYPO	MEG	N	HYPOLOBATED,Dec	NO	MM
14	BM-8/13	898282	Hema	19	F	GW,F	P	DA	2.6	5.8	100	55,000	1;3	HYPER	MIC/MEG	N	N	NO	EH+DM
15	BM-7/13	884565	Shanthi	30	F	Fwc	P	MA	3.2	2.8	56	20,000	1.2;1	NORMO	MIC	N	N,Inc	NO	EH+MicroM
16	BM-5/13	880148	Manjula	19	F	F,LW,LA	P	DA	2.6	4.2	88	42,000	1.2;1	NORMO	MIC/MEG	N	N	NO	EH+DM
17	BM-3/13	830024	Nazir Pasha	56	M	GW,PA	P	MA	3.3	4.6	104	91,200	3;1	HYPER	MEG,BF	GM	N	NO	EH+MM
18	BM-6/13	846052	Ananda	18	M	GW	HM,P,I	DA	2.6	2.1	99	10,000	1;5	HYPER	MIC/MEG	GM	N	NO	EH+DM
19	BM-2/13	872604	Mangala Gowri	50	F	GW,DS	P,E	DA	1.6	6.8	98	49,000	1;2	HYPER	MIC/MEG,BF	N	HYPOLOBATED	NO	EH+MM
20	BM-2/14	845698	Muniyamma	50	F	Fwc	P	NA	1.8	3.1	72	15,000	1;2	HYPO	MEG	N	N	NO	EH+MM
21	BM-1/14	975578	Venkatamma	50	F	Fwc,LW	P	MA	1.2	2.3	110	4,000	0.9;1	HYPO	MEG	N,Dec	N	NO	MM
22	BM-5/14	978068	Saraswathamma	78	F	GW,BA,Cough	P,E	MHA	4	5.1	75	22,000	1.1;1	HYPER	MIC/MEG	N	N	NO	EH+MicroM
23	BM-6/14	971424	Roopesh	60	M	BL,Kco ITP	P	NA,ATL	3.8	9.9	72	10,000	1;1	NORMO	NB	N	N	NO	EH+NM
24	BM-7/14	988983	Bagalappa	45	M	BA,F,Hematamsis	P	DA	2.2	4.4	98	10,000	2;1	HYPER	MIC/MEG	N	N	NO	EH+MM
25	BM-8/14	1001632	Sarojamma	50	F	Cough-bloody sptm	P	NA	3.2	3.5	76	40,000	3;1	NORMO	NB	N	N	NO	EH+NM
26	BM-9/14	1001634	Gowramma	32	F	BA,Fwc	P	DA	4	10	90	10,000	0.6;1	NORMO	NB	N	N	NO	EH+NM
27	BM-9a/14	10003027	Manjula	35	F	Fwc,Nasal bleed	P	DA	3.9	4.8	98	15,000	1.1;1	HYPO	NB	N	N	NO	NM
28	BM-10/14	1000858	Kaveri	21	F	Hematamesis,Mennoragia	P	DA	3	2.2	86	57,000	2;1	HYPER	NB	N	N,Inc	NO	EH+NM
29	BM-11/14	1002086	Gulab Jan	65	F	GW,Fwc,BA	P	MHA	2.9	2.2	86	100,000	1.1;1	HYPER	NB	N	N	NO	EH+NM
30	BM-12/14	10006869	Vandan Yadav	18	M	GW	P	DA	2.8	4.5	100	34,000	1;2	NORMO	MEG,BF	GM	HYPOLOBATED	NO	EH+MM
31	BM-15/14	226600	Suresh	40	M	GW,Hematuria	P	DA	2.9	6.4	89	30,000	1;1	NORMO	MIC	N	HYPOLOBATED	NO	EH+MicroM
32	BM-16/14	3509	Kavitha	21	F	Fwc,BA	P	DA	3.8	3.6	101	57,000	4;1	NORMO	MIC,BF,MF	N	N	NO	EH+MicroM
33	BM-18/14	10056	Venkataramappa	60	M	GW,BA,Malena	P	HE	4	9	92	60,000	1;2	NORMO	NB	N	N	NO	EH+NM
34	BM-19/14	12401	Ushashree	34	F	Fwc	HM,SM,P	MA	4	8	89	80,000	1;1	HYPO	NB	N	N	NO	NM
35	BM-23/14	23716	Manjunath	27	M	Fwc	P,L	DA	2.3	6.1	100	39,000	1;3	HYPER	MIC/MEG,BF	GM	HYPOLABATED	NO	EH+DM
36	BM-25/14	29421	Gayathri	30	F	Fwc,Jaundice	HM,P,I	MA	2.5	3	115	20,000	1;3	NORMO	MIC/MEG,BF	N	N	NO	EH+MM
37	BM-26/14	29407	Munivenkatappa	65	M	PA	HM,SM,P,I	MHAwEos	1.5	9.6	56	71,000	3;1	HYPO	MIC	N	HYPOLOBATED	NO	EH+NM+Eosin+Mast
38	BM-29/14	40572	Anjanappa	53	M	GW,Cough	P,I,E	MA	3.8	6	112	90,000	1;1	NORMO	MEG	N	N	NO	EH+MM
39	BM-30/14	46471	Govindappa	45	M	GW,F	HM,P,I	MHA	4	3	57	41,000	1;1.8	NORMO	MIC	N	N	NO	EH+MicroM
40	BM-31/14	47652	Srikanth	24	M	GW,F,Cough	P	AML	4	4.3	105	16,000	5;1	HYPER	MEG,BF	Blast 25%	Dec	NO	AML
41	BM-32/14	42585	Radha	19	F	GW,F,BL	P	MHA	2.3	4.2	59	57,000	2;1	NORMO	MIC	N	N	NO	EH+MicroM
42	BM-33/14	56863	Muniyappa	65	M	AltSensorium,BA	P,I	MHA	3.8	7.8	106	96,000	1.1;1	HYPER	MIC/MEG,BF	N	HYPOLOBATED,DG	NO	EH+DM
43	BM-34/14	46921	Radhika	26	F	BA,Fwc	P	MHA	2.1	4.2	56	80,000	2;1	NORMO	MIC	N	N	NO	EH+MicroM
44	BM-35/14	57551	Muniyappa.R	55	M	GW	b/L PE,P	DA	3	1.8	60	15,000	1;2	HYPER	MIC/MEG	N	HYPOLOBATED	NO	EH+DM
45	BM-36/14	67108	Shamshunissa	60	F	Fwc,PA,BL	P	DA	4	4.1	101	100,000	0.6;1	NORMO	MEG	N	N	NO	EH+MM
46	BM-37/14	70894	Murali Mohan	30	M	GW	P	DA	3.2	5.3	88	10,000	1;2	NORMO	NB	N	N	NO	EH+NM
47	BM-38/14	51447	Mugilamma	62	F	GW,F	P	MHA	4	3.4	93	28,000	2;1	HYPO	Red,MIC	DYSPLASIA	N,MastCells	NO	Pure Red Cell Aplasia
48	BM-39/14	78735	Venkateshappa	55	M	GW,Fwc	H,S,P	MA	2.3	4.8	59	80,000	1;2	HYPER	MIC/MEG	N	HYPERLOBATED	NO	EH+DM
49	BM- 40/14	10056	Venkatappa	60	M	Fwc,PA	P	NA,ATL	3.8	8	75	20,000	1;2	NORMO	NB	N	N	NO	EH+NM
50	BM-2/15	91199	Lakshmi Devamma	55	F	GW,Fwc,BA	P	DA	2	4.9	90	80,000	1;2.5	HYPER	MIC/MEG	N	N	NO	EH+MM
51	BM-3/15	93607	Seenappa	35	M	F,Cough,KOILONYCHIA	HM,SM,P,E	DA	3	4.8	82	100,000	0.6;1	HYPER	MIC	N	N	NO	EH+MicroM
52	BM-4/15	97770	Sham	28	M	Fwc	P	DA	3.4	4.1	99	90,000	1;2	HYPER	MEG,BF	N	N	NO	EH+MM
53	BM-6/15	98830	Mamatha	19	F	Fwc	SM,P	DA	3.5	4.6	98	80,000	2.5;1	NORMO	MEG	N	N	NO	EH+NM
54	BM-7/15	86646	Shantamma	45	F	GW,Fwc	P	NA	2.8	10	88	72,000	1;1	NORMO	NB	N	N,Dec	NO	EH+NM
55	BM-8/15	101852	Prema	19	F	F	SM,P	DA	3.2	3.3	111	25,000	3;1	HYPER	MEG,BF	GM	HYPOLOBATED	NO	EH+MM
56	BM-10/15	116234	Venkatamma	80	F	Nasal Bleed	P	DA	2.5	8.7	89	36,000	1.1;1	HYPER	MIC/MEG	GM	HYPOLOBATED	NO	EH+DM
57	BM-11/15	127208	Munirathnamma	35	F	Fwc,F	P	NA	4	7	86	100,000	2;1	HYPO	MEG	N,Dec	N,Dec	NO	MM
58	BM-12/15	125775	Narayanappa	43	M	Fwc,F,BA	HM,SM,P	NA	3.3	9	86	98,000	0.6;1	HYPER	MIC	N	N,Inc	NO	EH+MicroM
59	BM-13/15	130323	Venkataramappa	60	M	F,PA	P	NA	1	2	80	90,000	1.1;1	HYPO	NB	N,Dec	N,Dec	NO	NM
60	BM-14/15	13038	Deepa	24	F	GW,F,BA	SM,P	DA	3.3	8.2	98	100,000	1.5;1	NORMO	MEG	N	N	NO	EH+MM
61	BM-16/15	142053	Chandrasneha	38	F	Fwc,BA	P	NA	4	7.8	86	98,000	2.5;1	HYPER	MIC/MEG,BF	N	N,Dec	NO	EH+DM
62	BM-17/15	141703	Leelavathamma	65	F	GW,F,BA	P,E	DA	2.2	8.2	103	92,000	1;1	HYPO	MEG	N,Dec	N,Dec	NO	MM
63	BM-19/15	143204	Lakshmi Devamma	50	F	Blood in stools	P	DA	3.9	9.8	84	52,000	1.3;1	NORMO	NB	N	N,Inc	NO	EH+NM
64	BM-21/15	147283	Leelavathi	65	F	GW,F	P	MA	3.6	8.2	99	47,000	3;1	HYPER	MIC/MEG	N	N	NO	EH+DM
65	BM-22/15	155216	Shilpa	18	F	GW,F	P	DA	3.4	8	111	92,000	2.4;1	NORMO	MIC	N	N	NO	EH+MicroM
66	BM-24/15	170044	Nagesh	20	M	GW,F	HM,SM,P	MA	3.7	4.9	103	60,000	3;1	NORMO	MEG	N	N	NO	EH+MM
67	BM-25/15	180800	Padmamma	35	F	GW,F,PA	P	NA	3.7	7.1	76	60,000	3;1	NORMO	MIC/MEG	N	N	NO	MicroM
68	B/37/15	88185	Lakshmamma	56	F	F	P	NA	2.1	4.2	78	10,000	0	HYPER	MIC/MEG	N	N	NO	EH
69	B/1233/15	141703	Leelavathamma	65		F	P	MA	2	9.1	101	30,000	0	HYPO	NB,Dec	N,Dec	N,Inc	EOSI+	Hypoplastic+NM
70	B/879/15	130323	Venkataramappa	60	M	GW	SM	NA	1	6	86	48,000	0	HYPER	MEG	Imm Granulocт	Inc,Dys,HYPOLOBATED	EOSI+PLASMA+	MyelodysplasticMarrow